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INSULIN: ITS INTRADERMAL INJECTION*

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IN a previous article[1] dealing with the skin and the effect of insulin on the blood sugar content observations were described showing that the results of insulin injections in rabbits vary with the route of administration. Similar amounts of insulin, taken from the same container, produce different results on the blood sugar content depending upon whether it is injected subcutaneously, or intradermally.

After subcutaneous injection of insulin, a decrease in the blood sugar content was noted between the fifteenth and forty-fifth minute. The lowest figure obtained with 0.5 and 1 unit of insulin per kilo body weight in accordance with other observers, was about 50 per cent lower than the initial findings, but it rarely dropped to less than 0.45 mg. per cubic centimeter. This low level was maintained during the first two hours. Four hours after injection the decrease did not exceed 25 per cent of the original status and after six hours the initial figures of the blood sugar contents again prevailed. Similar curves were found to obtain in almost all of the cases examined where the drug had been administered subcutaneously, but a fundamental difference in the curve of the blood sugar content was observed after intradermal injection of 1 unit and of 0.5 unit of insulin. The sugar decrease began about the same time as after subcutaneous injection. The lowest point of the curve was also reached after a lapse of time corresponding to that in the foregoing tests. The striking difference became evident on comparison of the data obtained four hours after the intradermal injection. While the subcutaneously injected animals showed a decrease of about 25 per cent below normal at this time, the low blood sugar content noted

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after two hours in both kinds of animal tests was still observed in those injected intradermally. In other words the rabbits injected intradermally had a more prolonged blood sugar reaction than those treated subcutaneously with the same agent. Similar differences were demonstrated when determining the blood sugar content after a six hour period. At this time almost all the subcutaneously injected animals reached the normal level while the others still showed a decrease of about 25 per cent below normal.

Heretofore, there has been no explanation of the fact that insulin acts differently according to the route of administration. A detailed discussion of our findings and conclusions will be found in our previous articles. The question now arises as to the importance of the time of resorption. According to the findings mentioned above, the time of resorption, different though it may be in the skin and in the subcutaneous tissues, is not the cause of the difference in the blood sugar curves. The fact that, after both modes of administration, the lowest point of the curve was reached at almost the same time, contradicts any explanation by the resorption theory alone. If the specific substances reach the blood stream only through a slow process of resorption within the skin, then surely the onset of the reaction must be delayed. This not being the case the resorption theory cannot be accepted as an adequate explanation of this phenomenon.

Therefore it is concluded that the skin, or a special function of the skin, possesses the power of affecting the insulin reaction in the body in a way hitherto unknown. The skin as a special organ is closely related to the involuntary nervous system, as the body is related to the blood cells and to the serum, and is, therefore, capable of producing certain reactions in the body. It is known, for instance, that 0.1 c.c. of aolan, a nonspecific lactalbumin preparation, germ-free, toxin-free, and nonirritating, is an effective stimulant of the involuntary nervous system if given intradermally; on the other hand, subcutaneous, intramuscular or even intravenous administration of the same quantity of this agent is not followed by any measurable change in the organs controlled by the involuntary nervous system. These facts, briefly outlined on account of lack of space, make it seem highly probable that the effect of the intradermal administration on the insulin reaction is due to a special property of the skin, as yet unknown. If, as our tests show, the injection of a specific agent (insulin), intradermally, increases the effect of such agent as compared with its subcutaneous or intravenous employment, it must be concluded that an added feature of skin physiology has been discovered. Because of the great importance of this problem, as outlined above, it is desired to confirm and establish previous results. New studies along these lines have accomplished this, and it is our desire to present additional data of a confirmatory nature, thus providing an impetus to still further experimental work.

In our former experiments two animals were used for each test and the results obtained by intradermal and subcutaneous injection were carefully compared. It is known that animals, particularly rabbits do not respond uniformly to equal amounts of a given agent. The importance of this has not been given due consideration. But now the observation, that the response of the animal

organism varies with the route of administration, takes on a new value since it may be essential to establish the relationship between the skin and the involuntary nervous system on the one hand and the blood sugar content on the other; furthermore, it may be a factor in recognizing the rôle of the skin in the purely specific processes mentioned in a recent paper.[2]

In an article by Macleod and Orr[3] it is stated that rabbits, on the same diet and starved for twenty-four hours preceding the tests, cannot be considered strictly uniform since they vary in sex, age, and breed, and they also vary considerably in their susceptibility towards insulin, both with regard to effect on blood sugar and the incidence of convulsions. These variations have been recognized by us. It is our desire to present in detail the experiments whereby these factors have been controlled.

In their article Macleod and Orr have also given illuminating explanations of these effects which have been mentioned briefly by us. It is stated that the following must be taken into consideration: (1) Variability in the carbohydrate metabolism of different animals; (2) seasonable variations; (3) the development of an immunity to insulin.

As stated above, every possibility of such errors has been eliminated, as far as such elimination in biologic tests is possible. In the first place, variability in the carbohydrate metabolism of different animals as well as other individual differences between two animals, may be considered as nonexistent by using the same animals for the different tests after a week or two of rest. By conducting our experiments in this way, it was expected to avoid the other sources of error mentioned by Macleod and Orr in the second and third parts of their conclusions.

An interval of not more than two weeks between the tests in a single animal as well as the examination of the entire series within one month, may be considered a sufficient precaution to avoid mistakes by seasonal variations. The well-established statement by Macleod concerning the development of an immunity to insulin can evidently be ignored in *our* tests, because the same animals were not used more frequently than three or four times at the most.

Also by comparing these later tests of thrice used rabbits with the first tests in other animals, not previously used for insulin injections, there was no manifestation of an immunity to insulin in the less frequently used rabbits.

In Table I the results of experiments on seven different rabbits are given. Three of these animals were injected twice subcutaneously and twice intradermally; one was injected twice intradermally and once subcutaneously; the other three had one injection each by the subcutaneous and by the intradermal route. In the first column are recorded the results of the intradermal injections as shown by the blood sugar content in comparison with the initial blood sugar content. In the second column are found the corresponding values for subcutaneous injection. Each horizontal line shows two tests on the same animal by different methods of injection. Thus comparison may be made in the same animal, of the effects of the different routes of administration, and between different animals using the same method of administration.

Table II gives the same results by tabulating the percentage increase in the blood sugar content, only. These values are tabulated in the same manner as in Table I, showing in the first column the findings after subcutaneous insulin injections. The subdivisions in this table give the decrease in percentage in the second, fourth, and sixth hours as compared with the initial blood sugar content of each individual animal. In view of the fact that both tables deal with the same tests and that both are submitted only to facilitate a complete review of the data, our discussion of the findings will refer only to the second table. A graphic presentation of these results is found in Chart 1.[4]

TABLE I

SUMMARY OF EXPERIMENTS ON MODE OF INJECTION
THE BLOOD SUGAR CONTENT AFTER THE INJECTION OF INSULIN

	BLOOD SUGAR: MG. PER 100 C.C. HOURS AFTER INTRADERMAL INJECTION				BLOOD SUGAR: MG. PER 100 C.C. HOURS AFTER SUBCUTANEOUS INJECTION			
RABBIT NO.	0	2	4	6	0	2	4	6
58	152	62.5	63.5	47	123	45	100	129
58	115	39	49	67				
78	155	70	96	134	134	66	102	123
78	130	73	90	117	139	52	120	131
79	156	91	96	118	130	77	81	99
80	123	56	89	136	129	63	117	125
80	121	52	91	126	131	45	131	132
81	117	34	34	59	115	46.5	67	103
82	142	96	117	127	127	98	128	134
83	129	76	88	111	128	83	118	114
83	122	61.5	64.5	72	121	62.5	116	115
Average	132.9	64.6	79.9	101.3	127.7	63.8	108	120.5

TABLE II

SUMMARY OF EXPERIMENTS ON MODE OF INJECTION
PERCENTAGE CHANGE IN SUGAR CONTENT

RABBIT NO.	HOURS AFTER INTRADERMAL INJECTION			HOURS AFTER SUBCUTANEOUS INJECTION		
	2	4	6	2	4	6
58	-58.5	-58.0	-69.0	-63.5	-18.7	+ 4.9
58	-66.1	-57.4	-41.8			
78	-54.8	-38.3	-15.7	-50.8	-23.9	- 8.2
78	-43.8	-30.8	-10.0	-62.6	-13.7	- 5.8
79	-41.7	-38.5	-24.3	-40.8	-37.7	-23.8
80	-51.2	-27.6	+10.6	-51.2	- 9.3	- 3.1
80	-57.0	-24.8	- 4.1	-65.7	0.0	+ 0.8
81	-71.0	-71.0	-49.6	-59.7	-40.8	-10.4
82	-32.4	-17.6	-10.6	-22.8	+ 0.9	+ 5.5
83	-41.2	-31.0	-14.0	-35.2	- 7.8	-10.9
83	-49.6	-47.2	-41.0	-48.4	- 4.1	- 5.0
Average	-51.57	-40.2	-23.75	-50.07	-15.52	- 5.5

Table II.—Most of the findings are sufficiently clear to render needless an explanation of all the tests in detail. It is desirable, however, to examine the following individual tests on Rabbits 58, 81, 82 and 79. Rabbits 58 and 81 are both sensitive to the action of insulin. In Rabbit 81 convulsions ensued from an intradermal injection of 0.5 unit of insulin. In both animals the blood sugar level was quite low at the four and six hour bleedings following intradermal injection. Yet after the subcutaneous injection of the same amount of insulin

both recovered rapidly and were nearly normal at the end of the sixth hour. Rabbit 82, on the other hand, was not so sensitive to the action of this drug when given in the same amount and of the same lot number. This lack of reactivity was even more evident by the subcutaneous route of administration.

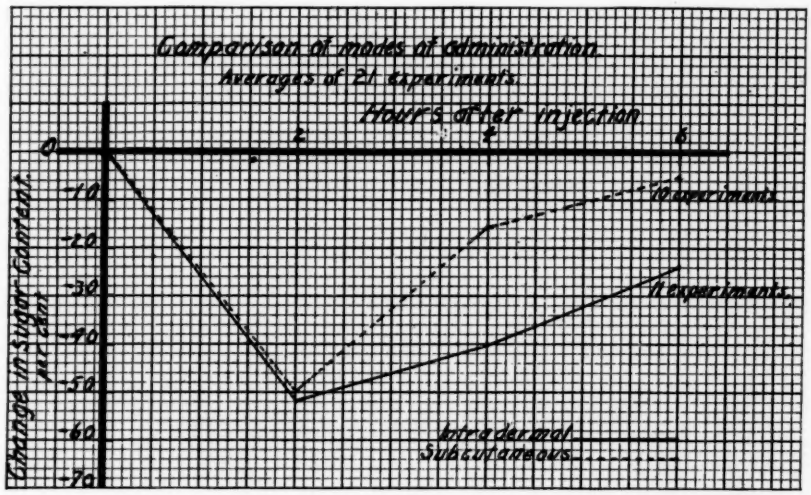


Chart 1.

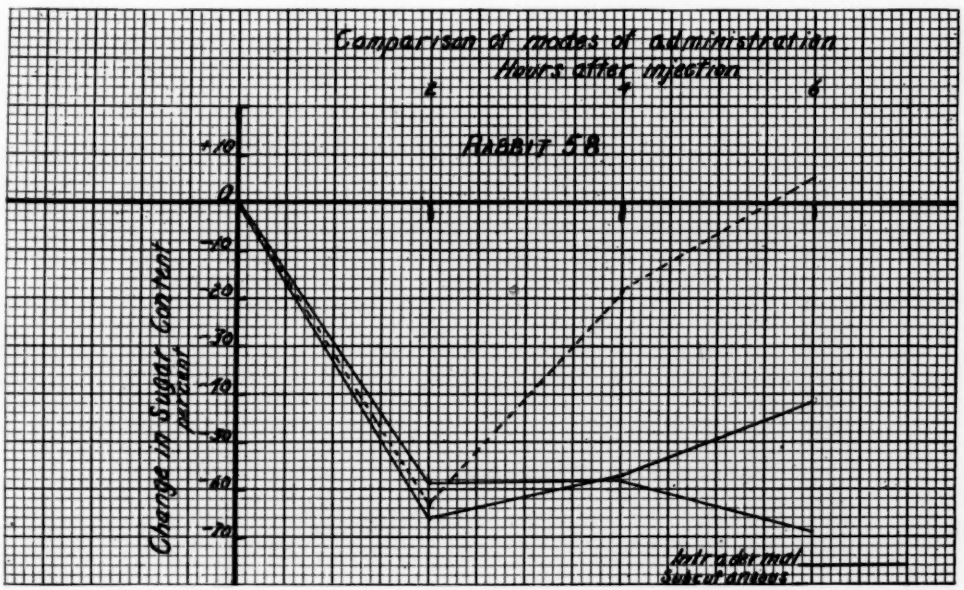
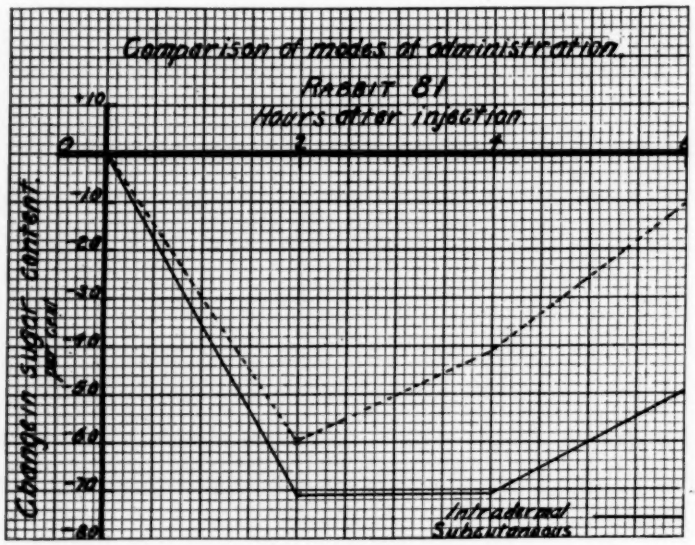


Chart 2.

Perhaps it should be noted here, that in other experiments to be published elsewhere, this rabbit was hypersensitive to the action of suprarenin and its derivatives. Still another type of animal is represented by Rabbit 79, little difference being observed between the results of the two methods of injection. Only one

animal of this type has been observed. A graphic illustration of these results is shown in Charts 3 to 5.

Chart 1 to 5.—Chart 1 gives a graphic presentation of the average changes in the blood sugar content after intradermal and subcutaneous injections, ob-



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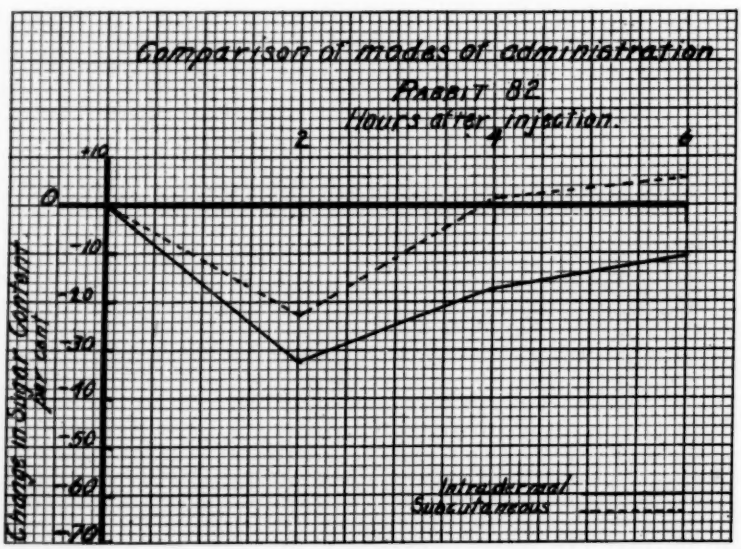

Chart 4.

tained from the above twenty-one tests in these seven animals and the variations from this average. These results may be regarded as additional proof of our previous findings and the variations of the effect of insulin.

Before summarizing the above findings, one more point must be mentioned. The amount of consideration which should be given to the effects of struggling

and its influence upon the results of experiments of this nature, has been shown in another paper,[4] viz.: properly controlled experiments are not affected by struggling as far as their results are concerned with the effect of insulin.

It must be concluded that after intradermal administration there is evident a prolongation of the effect of insulin on the blood sugar level of normal rabbits. These results are all the more important as they are obtained in accordance with

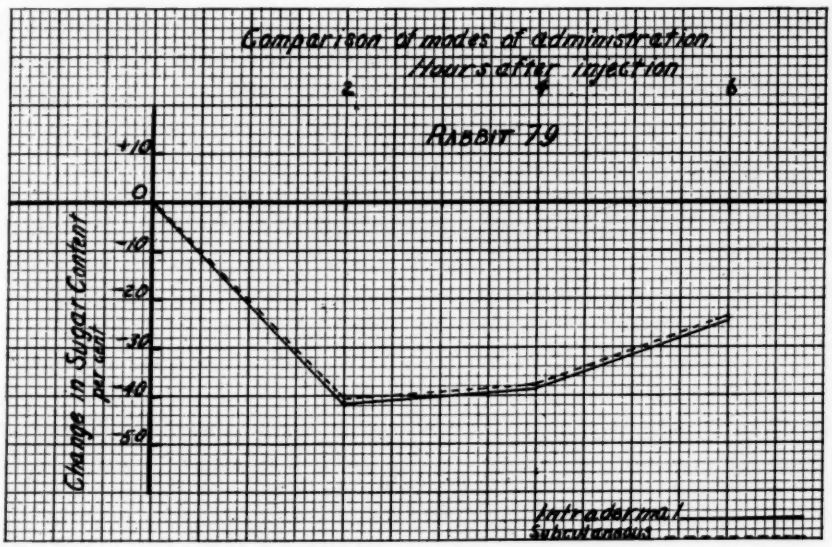

Chart 5.

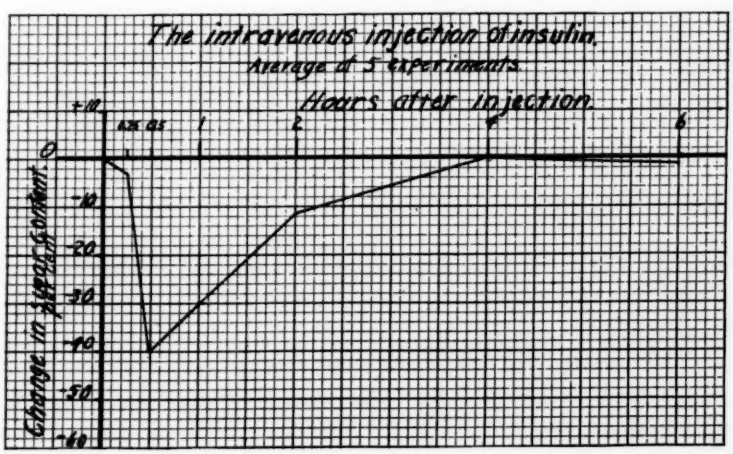

Chart 6.

the precautions prescribed by Macleod and Orr. Without going into further details, there is only one explanation possible, namely, that the skin as an individual organ is partly responsible for this effect. Of course while the factor of resorption plays an important part, it alone does not suffice to explain the phenomenon, since resorption constitutes only a special property of the skin which may influence the effect by its physiologic action. The following was

taken into consideration: the effect of insulin injections increases proportionately to the distance from the circulatory system at which the injection is made. It was, therefore, necessary to include a comparison of the effect of intravenous injection on the blood sugar content. These findings are given in detail in the paper mentioned above and show that the effect of insulin was not very marked when injected directly into the blood stream. The results following intravenous injection as given in Table III and Chart 6 show a decided difference as compared with previous figures. (As regards Chart 6, attention is directed to the fact that each single point of the curve represents a mean of the individual findings and that this average was not always obtained from an equal number

TABLE III

THE EFFECT OF INTRAVENOUS INJECTION OF 0.5 UNITS (PER KG. BODY WEIGHT) OF INSULIN UPON THE BLOOD SUGAR OF NORMAL RABBITS

RABBIT		UNIT PER KG.	TOTAL UNITS INJECTED	TIME AFTER INJECTIONS (HOURS)	BLOOD SUGAR CONTENT (MG. PER 100 C.C.)	CHANGE IN SUGAR CONTENT PER CENT
NO.	WT.					
80	2060	0.5	1.0	0.0	137	
				0.5	96	−40.0
				1.0	107	−21.9
				2.0	132	− 3.7
				4.0	138	+ 0.7
				6.0	138	+ 0.7
83	2450	0.5	1.2	0	121	
				1	76	−37.2
				2	97	−19.85
				4	123	+ 1.6
				6	122	+ 0.8
80	2075	0.5	1.0	0	138	
				2	113	−18.1
				4	126	− 5.8
				6	123	−10.9
81	2150	0.5	1.1	0.0	120	
				0.25	124	+ 3.3
				2.00	91	−24.2
				4.00	125	+ 4.2
				6.00	127	+ 5.8
82	2250	0.5	1.1	0.0	129	
				0.25	117	− 9.3
				2	141	+ 9.3
				4	129	0.0
				6	127	− 1.6

of animals. The number of determinations represented by every point of the curve is readily seen in Table III.) One-half hour and one hour after intravenous injection there was a marked decrease in the blood sugar content; however, this level is but little below the initial findings and lasted only for a short time. At the end of the two hour period the blood sugar decrease had greatly diminished and corresponds to the four hour level after subcutaneous, and to the six hour level after intradermal injections. Four hours after intravenous injection the blood sugar content was almost normal. Perhaps the most important of these findings is that the blood sugar decrease thirty minutes after injection was comparatively small. Therefore, insulin, which enters the blood stream in highly concentrated form, is not able to produce a decrease in the blood sugar

content in proportion to this higher concentration. This observation is sufficient proof that the factor of resorption alone does not explain our findings. We have thus by tests of intravenous administration supported our former conclusion that the effect of insulin injections increases proportionately to the distance from the circulatory system at which the injection is given. In other words, intravenous injections are the least effective, subcutaneous injections are considerably more so and the intradermal mode of administration is by far the most effective. These findings suggest a comparison with earlier experiences with nonspecific agents. Their effect, especially when nontoxic and nontissue-irritating preparations are used, is also greatly enhanced by the intradermal method of injection; while equal doses administered intravenously are ineffective, and it has been definitely determined that this increased effectiveness is due to the close relation of the skin to the involuntary nervous system.

It was our aim to exclude certain sources of error cited by Macleod and Orr as also certain factors which might influence the final results, viz.: struggling of the animals. Furthermore, it was our intention to broaden the basis of our investigations by conducting the same tests in the same animals thus basing our knowledge upon results secured with identical biologic conditions. A special series of experiments, differing in the method of attack from those in this paper, will be necessary to carry on these studies; the foundations for which have been laid by this and previous papers.

CONCLUSIONS

1. The effect of insulin varies with the route of administration. The effect of insulin injections upon the blood sugar content of normal rabbits is markedly increased if the intradermal method of administration is employed; subcutaneous injections of the same amount of insulin into the same as well as into different animals are less effective.

2. A comparison of intradermal, subcutaneous, and intravenous injections of equal amounts of insulin shows that their effectiveness decreases in the order mentioned.

3. The degree and the period of effectiveness does not depend upon the rapidity of resorption, but upon a hitherto unknown factor, very probably related to the action of the involuntary nervous system.

4. This property seems to be inherent in the skin rather than in the subcutaneous tissues or in the body fluids.

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STUDIES ON THE CARBOHYDRATE METABOLISM OF INFANTS*

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PRELIMINARY COMMUNICATION

THE present paper is entirely in the nature of a preliminary communication. It is based on between five and six hundred blood sugar determinations which have been made largely in an attempt to lay the foundation for future work, which had been planned at the outset. A number of interesting facts have so far been found which we think are of sufficient importance as to justify their presentation at this stage of the work.

Last year a study was made in this laboratory of the acidity of infants' stools[1] and the interesting and rather surprising observation made that, in certain cases, loose acid diarrheal stools could be changed to constipated alkaline ones, by the use of a soured milk diet with a carbohydrate content as high as twenty per cent. When it is remembered that the amount of acid in the stools is largely, if not entirely dependent on the fermentation of carbohydrate in the intestinal canal, the above finding is most surprising. A number of theories were advanced in an attempt to explain this, but little proof could be produced in their support. Partly as a result of this, the present study on the metabolism of carbohydrates in infancy was planned. Two main objects were in view,—first to determine the rate of absorption of various carbohydrates in different mixtures from the gastrointestinal tract, and second to determine the ability of the infant to handle carbohydrate after it reached the blood stream.

The first two hundred determinations were made by the well-known Schaffer-Hartmann[2] method. This method requires two cubic centimeters of blood for each determination. It was soon seen that repeated determinations could not be made readily on the same infant on account of the loss of blood entailed. Consequently the following procedure was devised which requires only 0.2 c.c. of blood and gives results which are usually accurate within 5 per cent.

Procedure for the determination of the blood sugar with the use of 0.2 c.c. of blood:

Two-tenths c.c. of blood is measured into a 15 c.c. centrifuge tube containing 1.4 c.c. of water. This is thoroughly mixed. There is then added 0.2 c.c of 10 per cent sodium tungstate and 0.2 c.c. of ⅔ normal sulphuric acid and the contents again thoroughly mixed. This precipitates the protein. The tube is then centrifuged for five minutes at high speed which drives all the precipitate to the bottom of the tube and leaves about 1.5 c.c. of clear supernatant fluid. One c.c.

*From the Wards and Nutritional Research Laboratories of the Hospital for Sick Children, and the Department of Pediatrics of the University of Toronto.

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of this fluid is measured into a test tube 1.3 mm. in diameter and 12 cm. long. To this is added 1 c.c. of the Schaffer-Hartmann blood sugar reagent and the contents mixed. A small funnel is inserted in the tube which is then placed in a boiling water-bath for exactly fifteen minutes. The tube is cooled in water.

TABLE I

AMOUNTS OF GLUCOSE CORRESPONDING TO IODINE USED FOR VALUES BELOW 0.100 PER CENT

0.005 N. IODINE REQUIRED TO OXODIZE CUPROUS OXIDE	GLUCOSE IN BLOOD
c.c.	per cent
3.1	0.100
2.8	0.095
2.6	0.090
2.4	0.085
2.2	0.080
2.0	0.075
1.8	0.070
1.6	0.065
1.4	0.060
1.2	0.055
1.0	0.050
0.8	0.045
0.6	0.040
0.4	0.033

TABLE II

VALUES FOUND ON KNOWN THEORETICAL SOLUTIONS OF DEXTROSE USING THE SCHAFFER-HARTMANN TABLE

THEORY	FOUND
Per cent	Per cent
0.400	0.403
0.400	0.420
0.200	0.207
0.200	0.206
0.200	0.206
0.125	0.125
0.100	0.098
0.100	0.099

TABLE III

COMPARISON OF THE AMOUNT OF BLOOD SUGAR FOUND BY THE SHAFFER-HARTMANN METHOD AND THE MODIFIED PROCEDURE

NO.	SHAFFER-HARTMANN METHOD	MODIFIED PROCEDURE
	per cent	percent
1	0.053	0.055
2	0.88	0.090
3	0.093	0.098
4	0.104	0.104
5	0.130	0.130

One c.c. of 5 per cent sulphuric acid is added, a drop of one per cent starch solution as indicator, and the contents titrated with N/500 sodium thiosulphate delivered from a microburette calibrated in 0.02 c.c. A blank determination is made on one c.c. of the reagent with one c.c. of water added in place of the blood filtrate. The number of cubic centimeters of N/500 sodium thiosulphate used

with the blood filtrate (about 3.4 c.c. in normal infants) is subtracted from the amount used with the blank (about 3.9 c.c.). The value obtained is multiplied by five which gives a figure which corresponds to that which would have been obtained by the original Shaffer-Hartmann method. From their table the corresponding blood sugar values are obtained for all concentrations of 0.1 per cent or over. Below this concentration it was found that a definite but constant error occurred when their table was used. This necessitated the construction of a new table for values below 0.1 per cent. These figures are given in Table I.

The values found on known theoretical solutions of dextrose are given in Table II and a comparison of the amount of blood sugar found by the standard Schaffer-Hartmann method and the modified method are given in Table III.

It is seen that the procedure here used, consists of the Schaffer-Hartmann method in its entirety, only in the first stage one-tenth of the amount of blood

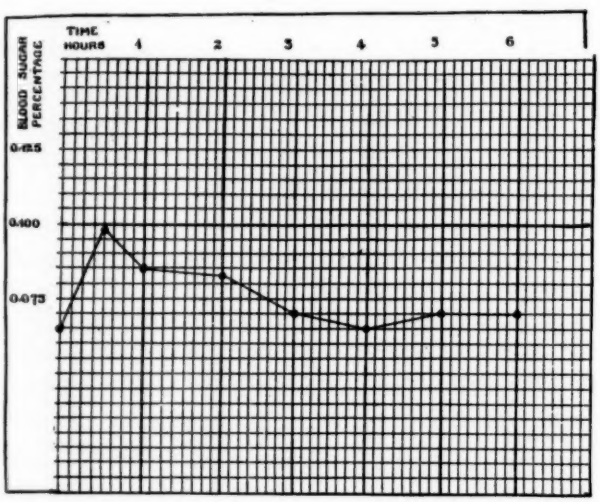

Chart 1.—Baby D. K., weight 8 pounds, 12 ounces. Blood sugar curve after feeding 4 ounces of the mixture of lactic acid, milk 10 ounces, water 10 ounces, and corn syrup 1 ounce.

is used, while in the latter stage one-fifth the amount of filtrate is used and the final titration multiplied by five.

The average blood sugar found in twenty approximately normal infants was 0.090 per cent. The lowest value found was 0.081 per cent and the highest 0.100 per cent. These infants were not perfectly normal but as nearly normal as can be obtained in an active service of a children's hospital. The blood sugar was determined in twelve cases of marasmus. These infants were all only two-thirds to one-half of their normal weight for their age. The average value found was 0.077 per cent. Only two of the infants had blood sugar values below 0.060 per cent.

The time in which the blood sugar returned to its usual level after an average feeding was studied in six cases. They were all infants suffering with marasmus. Two infants were gaining in weight, two were losing and two were stationary in weight. The same type of curve was found whether the infant was gaining or losing The average initial blood sugar content was 0.070 per

cent. The average maximum height reached was 0.102 per cent, which is an increase of 46 per cent over the initial value. The maximum concentration of blood sugar was reached in half an hour in four cases and in one hour in two cases. At the end of two hours the values were usually slightly above the original figures, while at three hours they were approximately the same as those obtained at the initial determination. The blood sugar then remained nearly stationary for the next three hours with a slight tendency to rise. No

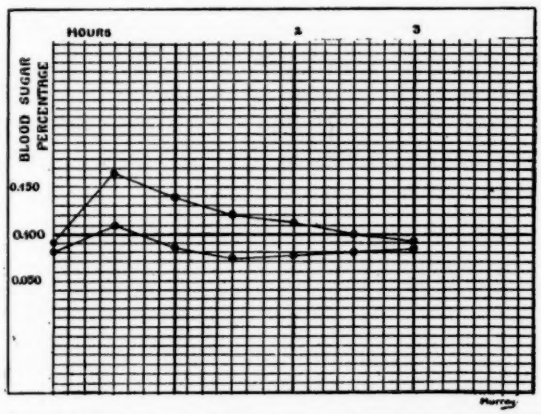

Chart 2.—The lower line represents the curve of the average blood sugar values found after the interstitial injection of five per cent glucose. The upper line represents the curve of the average blood sugar values found after the interstitial injection of 10 per cent glucose. The infants were approximately normal.

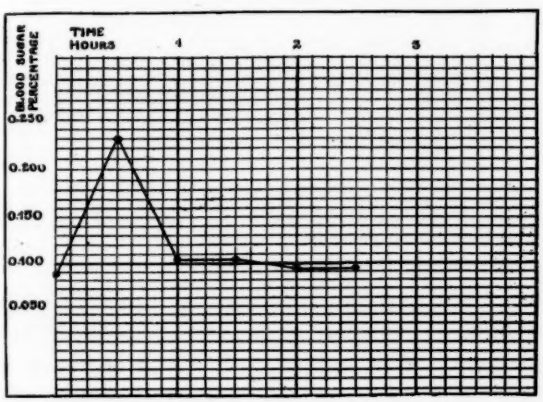

Chart 3.—Illustrates the rapidity with which the blood sugar may drop in certain normal infants after the interstitial injection of 10 per cent glucose.

determinations were made more than six hours after the feeding. The feedings consisted of lactic acid milk ten ounces, water ten ounces, and corn syrup one ounce. The carbohydrate content was 8.5 per cent and the amount given was 0.4 ounces per pound of body weight. The curve of values found with one infant is shown in Chart 1.

Blood sugar determinations were made after the oral administration of one ounce of the various carbohydrates in common use. Entirely different curves were obtained with different carbohydrates but as the results were so variable we do not feel justified in presenting them at this time.

The blood sugar curves, after the administration of glucose interstitially, were studied in sixteen cases. Three infants received 5 per cent glucose, eleven 10 per cent and two 15 per cent. A preliminary fasting blood sugar estimation was made, followed by half-hourly determinations for three hours after the conclusion of the injection. In most instances it required from fifteen to thirty minutes to give the interstitial injections, although occasionally it required as much time as forty-five minutes. Approximately 10 c.c. of solution per pound of body weight was given. With the five per cent glucose the initial value in three cases was 0.078 per cent. The highest value was obtained on the average in half an hour after all the glucose was given. The average value was 0.110 per cent, a percentage increase of forty. The surprising observation was made that the blood sugar level returned to the original value in an hour in two cases, and an hour and a half in the third case. In Chart 2 is a plotted curve of the average values obtained.

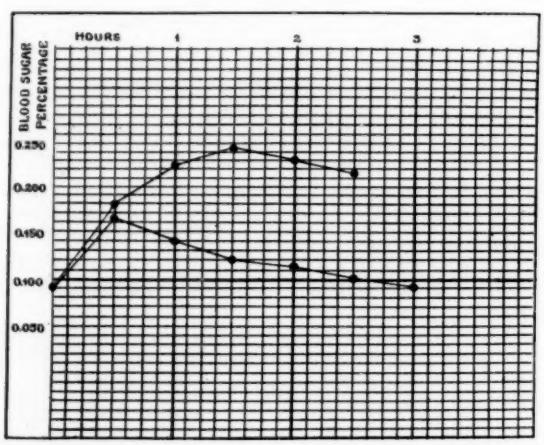

Chart 4.—The upper line represents the curve of the blood sugar values found after the interstitial injection of 10 per cent glucose in an infant suffering from acute intestinal intoxication. For comparison the lower line gives the average values found in normal infants after the interstitial injection of 10 per cent glucose.

Ten per cent glucose was given interstitially in eleven cases; seven of these infants were approximately normal. The average initial value in these seven cases was 0.090 per cent. The highest value was obtained on an average in slightly over half an hour after all the glucose was given. The average of the highest values was 0.165 per cent. The time required to return to the original figure varied from one hour to three hours. In Chart 2 is a plotted curve of the average values obtained with the seven approximately normal infants. In some of these cases the drop in blood sugar concentration after the interstitial occurred very rapidly. This is illustrated in Chart 3.

In the four abnormal cases the blood sugar curve dropped more slowly. These infants suffered from acute intestinal intoxication, lobar pneumonia, tetany with nasopharyngitis, and Potts' disease. Attention is directed only to the results obtained with the infant which suffered from intestinal intoxication, as the values with the other cases more nearly approached the normal figures. In Chart 4 is plotted the curve obtained in this case with the average values

of the seven normal cases for comparison. Reference will be made to this case later.

In regard to the use of fifteen per cent glucose, interstitially, the values found in two normal infants did not differ materially from those found with the use of the ten per cent solution.

Glucose was given intravenously to ten infants. Five of the infants were approximately normal while the other five were in poor condition. The pathologic conditions present in the infants in the latter group were fermentative diarrhea and mild intestinal intoxication. A ten per cent solution of glucose was employed and the infants were all given approximately 10 c.c. per pound of body weight. In Chart 5 is shown the average curves of the blood sugar content of both the normal and abnormal infants. It is to be noted that the blood sugar in the five normal infants returned to

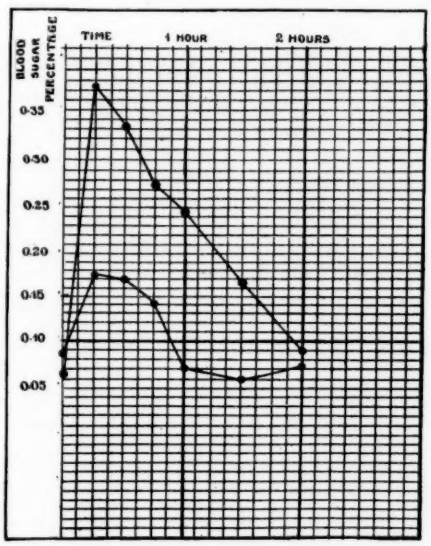

Chart 5.—The upper line represents the curve of the average blood sugar values obtained after the intravenous injection of 10 per cent glucose to five infants suffering from acute intestinal intoxication and fermentative diarrhea. The lower line represents the curve of the average blood sugar values obtained after the intravenous injection of 10 per cent glucose to five approximately normal infants.

the original level in one hour's time; in fact the value was actually below the initial value (initial value 0.082 per cent, one hour later 0.071 per cent). The maximum height of the curve was 0.174 per cent. In contrast to the normal infants the blood sugar concentration in the five abnormal cases did not return to the original level within two hours. The height of the curve was almost double that obtained with the normal infants and at one hour after the injection, the average concentration was 0.247 per cent instead of less than 0.100 per cent, as was found with the first group of infants. One and a half hours after the injection the concentration was still 0.167 per cent.

It appears that infants acutely ill with the above pathologic conditions are not able to utilize the glucose given either intravenously or interstitially with the same rapidity as the normal infant. This of course at once brings up the

question, is there a deficiency of pancreatic function in these cases? The most constant pathologic finding in this type of case, however, is a large fatty liver. This condition is also found in cases of intoxication following anesthesia and it is known that this can frequently be prevented by the preliminary administration of carbohydrate. It appears that when glycogen is deposited in the liver it protects the organism from the effects of an intoxication. The question arises, will glycogen be deposited in the livers of these infants if insulin is given with the glucose? This is not at all certain. Professor Macleod[3] has found, that in the case of a normal animal, the administration of insulin and glucose is not followed by the deposition of glycogen in the liver, but such deposition does occur in a diabetic animal.

At the present time we are continuing our studies on this most interesting aspect of carbohydrate metabolism. We hope that some valuable information

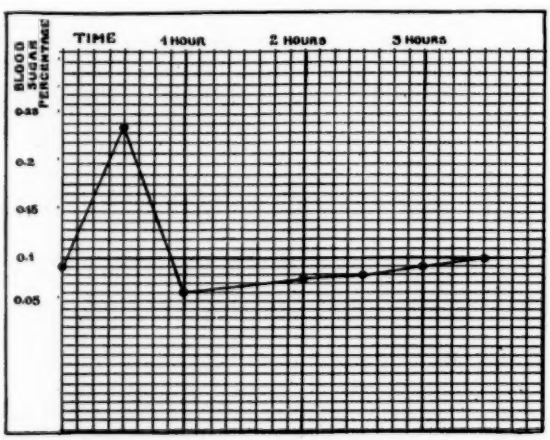

Chart 6.—Blood sugar curve obtained in Baby E. S. after the intravenous injection of 10 c.c. per pound of body weight of 10 per cent glucose. It is to be noted that at the end of one hour the blood sugar concentration is less than before the glucose was introduced. The blood sugar concentration then slowly increased. This curve might be taken to indicate an overproduction of insulin under the stimulus of the injected glucose.

may be obtained by including in this work the determination of the respiratory quotient, and also the glycogen and fat content of the liver of any suitable cases coming to autopsy.

SUMMARY

A procedure has been devised whereby blood sugar determinations may be made with the use of only 0.2 c.c. of blood. The procedure is based on the Shaffer-Hartmann method. The results obtained are usually accurate within 5 per cent.

The average fasting concentration of blood sugar in twenty approximately normal infants was found to be 0.087 per cent. In twelve cases of marasmus, the average concentration was 0.070 per cent. Values as low as 0.050 per cent were obtained in the latter group.

When 5, 10, and 15 per cent solutions of glucose are given interstitially to normal infants the blood sugar curve reaches its height about half an hour after

the injection is given. This rapidity of absorption is of practical value when an intravenous injection cannot be given readily.

The rise in blood sugar concentration in normal infants is more marked after the interstitial injection of 10 per cent glucose than after the interstitial injection of 5 per cent glucose. The fall in the blood sugar curve after interstitial injections of glucose occurs with remarkable rapidity.

The intravenous injection in normal infants of large amounts of glucose; i. e., one gram of glucose per pound of body weight, is followed by a rapid diminution of the blood sugar concentration, after the preliminary rise. One hour after injection, the concentration may be actually less than that found before the injection.

Infants with conditions such as acute intestinal intoxication and fermentative diarrhea are unable to utilize glucose introduced parenterally with the same rapidity as normal infants. A study of the effect of insulin in these cases is in progress.

Attention is again directed to the tremendous ability of the normal infant to utilize glucose introduced parenterally.

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DIETETIC MANAGEMENT OF CARDIAC, VASCULAR, AND RENAL DISEASE*

By Joseph R. Darnall, M.D., Capt., Medical Corps, U. S. Army

PROPER dietetic care of the cardiac, vascular, and renal invalid is largely dependent on the diagnostic and therapeutic judgment of the attending physician, and the diets required may vary widely for different patients. Numerous factors contribute to the undesirability of arbitrarily fitting certain type diets to certain type diseases. These factors include a careful consideration of the functional capacities of the digestive organs, heart, vessels, and kidneys, which vary in different patients, and may even vary greatly in the same patient within short periods of time. Individual likes and dislikes, idiosynerasy, and tolerance for certain foods must be considered.

Many functional cardiac disorders, as well as mild, well compensated valvular disease, may require no dietary regulation. In cases, however, where the circulation is maintained only by excessive cardiac effort, as in chronic hypertension, and in long standing or extensive valvular disease, an effort should be made through diet, to lessen the cardiac burden. This dietary regulation is even more important, in cases of actual myocardial degeneration.

ORGANIC HEART DISEASE

The processes of digestion, assimilation, and excretion demand considerable energy, and if not properly controlled, may add to the burdens of a failing heart. Passive congestion of the digestive organs necessitates a careful selection and limitation of food and drink. In general, foods most readily digested, and producing waste products most readily excreted, should be chosen. The following rules, then, should be observed in regulating the diet in organic heart disease:

1. The diet should be relatively dry, and easily digestible.

2. Foods causing flatulence should be avoided. These foods will vary in different individuals, but in general, beans, peas, cabbage, and starchy foods are more liable to produce flatulence.

3. The heaviest meals should be at noon, and breakfast. Supper should be light. Meals should be about five hours apart, and no solid food taken between meals.

4. Fluids should be restricted to 1500 c.c. if edema is absent. In the presence of slight edema, fluids should be limited to 1000 c.c. If edema is marked, with effusion into the serous cavities, fluids should not exceed 800 c.c. If the edema persists or increases, the Karell diet may be tried. This consists of giv-

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ing 200 c.c. of skimmed milk at 8 A.M., 12 NOON, 4 P.M., and 8 P.M., for from five to seven days. The diet is then gradually increased, adding first toast, then vegetables, one or two eggs, and finally a little meat.

5. Salt should be restricted, as it increases desire for liquids, and also withdraws fluid from the tissues into the blood stream, thus increasing the volume of the blood, and adding to the cardiac burden.

VASCULAR HYPERTENSION

The following dietary instructions are given to patients with vascular hypertension. Adherence to this regime is beneficial for all individuals past fifty years of age, whether or not they are suffering from increased vascular tension:

1. Restrict fluids moderately, taking about 1500 c.c. daily. Drink a glass of hot water just before breakfast, to cleanse the stomach and upper bowel and stimulate peristalsis.

2. Limit coffee or tea to one cup daily, preferably at breakfast.

3. Avoid overeating. A general quantitative reduction in diet is important. If a general reduction in diet aggravates or induces constipation, harmless bulk in the form of bran, fruits, and green vegetables may be added.

4. Avoid salty foods, such as ham, bacon, and salt fish. Do not put salt on food.

5. Eat meat (fish, flesh, or fowl), only once a day, and then only a small portion.

6. Do not eat more than one egg a day.

7. Avoid all highly seasoned foods, spiced foods, and restrict sweets.

8. Diet should be composed largely of green vegetables. potatoes, fruits, cereals, rice, hominy, spaghetti, bread (especially whole wheat and graham), and limited deserts.

Myocardial insufficiency, or impending decompensation will necessitate modification of the diet, along the lines indicated for heart disease.

CHRONIC INTERSTITIAL NEPHRITIS

In these cases, delicate judgment may be required to determine the fluid volume to be allowed. Too much fluid will burden the heart, and cause strain on damaged vessels; while too little fluid will impair the dilution and excretion of toxins through the kidney. In this disease the kidneys are unable to excrete concentrated urine.

The fluid should average between 1500 c.c. and 2000 c.c. daily, if there is not excessive hypertension,—that is, if the systolic pressure is below 200 mm. Protein should be restricted to 50-80 gm. daily, and salt should not exceed 5 gm. The total calories should be limited to 2000-2500 daily. The diet, then, should be reasonably balanced, easily digestible, with moderate protein and salt restriction, and a general quantitative limitation. It is most important to preserve heart muscle nutrition and compensation, and any modification of the diet will depend largely on the efficiency of the myocardium.

CHRONIC PARENCHYMATOUS NEPHRITIS

A normal fluid intake is desirable, if there is no edema. If edema is present, allow only 1000 c.c. fluid daily, and decrease or increase, as the variation in edema indicates. Forcing fluids in these cases sometimes promotes diuresis and disappearance of the edema, apparently due to the fact that the kidney can excrete salts in dilute solution, but cannot eliminate concentrated salt solutions. It is believed that forcing fluids should not be tried unless restriction of fluids fails to bring about a definite subsidence of the edema. Protein should be limited to 50-80 gm. daily. Salt should be markedly restricted, and obstinate cases of edema may require a strict salt-free diet, no salt being used even in the cooking of the practically salt-free foods. (Occasionally restriction of salt increases the edema, and 5-10 gm. of salt in such cases may act as a diuretic.)

Epstein advanced a theory that certain cases of edema with albuminuria differ from the usual picture of chronic nephritis, and that in these cases the edema is due to depletion of albumin, and he, therefore, advocates a high protein diet. These cases suitable for high protein diet are said to present an alteration of the normal ratio of albumin to globulin in the blood, and a high blood cholesterol. He advocates 120-240 gm. of protein, 20-40 gm. of unavoidable fat, and 150-300 gm. of carbohydrate. Consensus of conservative opinion is that cases responding favorably to such treatment must be extremely rare, and that while in some cases disappearance of edema may indicate improvement, the blood nitrogen will be increased, and the phthalein excretion diminished, as has been demonstrated in cases checked with blood nitrogen determinations and phthalein excretion tests.

ACUTE NEPHRITIS

In general, the diet should be adequate for the needs of the patient, and yet demand as little excretory function as possible, on the part of the kidney. The following general rules should be observed:

1. Marked restriction of protein and salt, and moderate restriction of fluids. The protein should be reduced to 20-30 gm. and the salt to 2-3 gm. Marked edema may call for strict salt-free diet. Fluids should be reduced to 1000-1500 c.c.

2. A milk diet may be given, or if greater reduction of protein and salt is deemed advisable, a diet of fruit juices, thin gruels, crackers, toast, rice, and potatoes may be substituted.

3. If much fluid is lost by vomiting, diarrhea, or sweating, it must be replaced, to dilute the toxins and facilitate the excretion of waste products.

4. After the acute symptoms have subsided, the diet may be increased, depending for selection of foods, on the presence and relative severity of nitrogen or salt retention.

CONCLUSION

No attempt has been made to give detailed advice or set dogmatic rules, but only to present general plans for the logical dietary treatment for cases of cardiocirculatory impairment and renal insufficiency. It has been the author's inten-

tion to outline certain dietary principles which may serve as a flexible guide to the dietary management of these closely associated and often overlapping pathologic conditions.

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STUDIES ON THE PNEUMOCOCCOLYTIC ACTIVITY OF THE BILE FROM EXPERIMENTAL BACTERIAL CHOLECYSTITIS OF RABBITS*

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REPORTS of the routine bacteriologic examination of bile have included, from time to time, the isolation of the pneumococcus. These reports with the knowledge that, as a rule, bile is highly bacteriolytic for the pneumococcus have been accepted with more or less reservation; for, in the instances reported, no explanation is offered why it is possible for the pneumococcus to exist.

This institute[1] has recovered on three occasions, the pneumococcus from human bile, and like findings in a similar number of cases have been reported by Richey[2]; one isolation from a gall bladder in which the bile had been supplanted by purulent material, and in the remaining two, the pneumococcus was recovered from the gall bladder wall, the bile, and the nucleus of a calculus. Piersol and Bockus[3] refer to the isolation of the pneumococcus from bile, but in their own investigations did not find it.

Naturally in view of these findings the thought suggested itself,—is the bile from gall bladders infected with the microorganisms commonly found in cholecystitis, such as the streptococcus, Staphylococcus aureus, Staphylococcus albus and B. coli bacteriolytic for the pneumococcus? In general, the procedure adopted to meet this question was to produce a cholecystitis in rabbits by the intracystic injections of the above-mentioned microorganisms, and after an interval of three weeks, determine the pneumococcolytic activity of the bile. The microorganisms selected were all isolated in this institute from pathologic human bile except the pneumococcus, and were cultivated on 5 per cent human blood agar in tubes inoculated over the entire agar surface from a hormone broth culture, and incubated at 38° C. for twenty-four hours. The resulting growth was suspended in physiologic salt solution so that each c.c. represented two billion microorganisms. Cultures were made of these suspensions to be assured of their viability and intracystic injections of 0.2 c.c. made in the respective rabbits. The rabbits varied in weight from two to two and one-half kilos.

The direct injection of the gall bladder was made by following the technic of Beckwith[4] which is briefly as follows: After securing the animal, the site of operation of the abdomen was prepared under rigid aseptic procedure. Ether was administered and an incision approximately one inch long was made to one side of the median line with its anterior end at the base of the sternum. With retractors and sponges, the gall bladder was revealed in its cleft. With smooth blunt forceps the gall bladder was gently drawn forward and injection of the bacterial suspension made with an ordinary No. 27 gauge needle. No leakage

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or bleeding occurred as the amount injected, 0.2 c.c., did not cause any appreciable intracystic pressure. The abdominal incision was then sutured and the wound treated with general surgical asepsis.

By this technic, of all rabbits injected, only one died as a direct result of the operation and this rabbit never fully recovered from the anesthetic. Three weeks later, all infected rabbits and two apparently normal rabbits were chloroformed, the bile withdrawn and placed in sterile test tubes. To each bile sample was added a two billion per c.c. suspension of pneumococci, Type I, in the proportion of 0.2 c.c. to every cubic centimeter of bile and incubated for one hour in a water-bath at 38° C. At one-half hour intervals 0.1 c.c. was removed and cultured on 5 per cent human blood agar plates. Table I gives the results of this work in detail.

TABLE I

PNEUMOCOCCOLYTIC ACTIVITY OF BILE FROM INFECTED GALL BLADDERS OF RABBITS

RABBIT NO.	CHOLECYSTITIS PRODUCED BY INTRACYSTIC INFECTIONS	PNEUMOCOCCOLYTIC ACTIVITY OF BILE	
		ONE-HALF HOUR	ONE HOUR
47	Streptococcus mitis	Absent	Absent
50	Streptococcus mitis	"	"
51	Staphylococcus aureus	"	"
52	Staphylococcus aureus	"	"
53	Staphylococcus albus	"	"
22	Staphylococcus albus	"	"
12	B. coli communior	"	Present
62	B. coli communior	"	"
37	Uninfected control	Present	"
84	Uninfected control	"	"

From Table I, it will be observed that bile from rabbit gall bladders infected experimentally with the Streptococcus mitis, Staphylococcus aureus, and Staphylococcus albus loses its pneumococcolytic action; while in bile from B. coli communior infected gall bladders, the pneumococcolytic action, while somewhat delayed, is present at the end of an hour. Compared with the action of normal rabbit bile where the pneumococcolytic action was prompt, these results are quite in contrast.

Injection of 0.2 c.c. of a two billion per cubic centimeter suspension of pneumococci into the gall bladder of a living rabbit, infected six weeks previously in the same manner with B. coli communior, confirmed these results in so far as the experiment was carried. In brief, a cholecystitis was produced by intracystic infection with B. coli communior isolated from the colon of a rabbit dying during an epidemic of infectious diarrhea; six weeks later the gall bladder was again exposed and 0.2 c.c. of a two billion per cubic centimeter suspension of pneumococci injected. One-half hour later the bile was withdrawn in a sterile capillary pipette and cultured on 5 per cent human blood agar plates and incubated at 38° C. for forty-eight hours. Many colonies of pneumococci and B. coli communior developed. The bile of two living normal rabbits, receiving intracystic injections of a similar amount of pneumococci suspension, showed no growth on culturing after the same length of time.

Other types of microorganisms, besides B. coli, were not used in the above experiment because it was felt that the *in vitro* experiments outlined in Table I,—the proportion of bile to the amount of pneumococcus suspension—was controlled and the results more dependable.

On autopsy, all the rabbits showed marked adhesions of the gall bladder, the adjacent lobes of the liver, and in several rabbits these organs were adhered by somewhat fibrous bands to the anterior wall of the stomach. The gall bladder wall was considerably thickened. The bile varied in color from faint yellow with a green tinge, to dark olive green and from slight to moderate turbidity. No evidences of coccidiosis were noted in any of the rabbits.

The microscopic examination of sections of the gall bladder of each rabbit, stained by the Gram-Weigert method, revealed many bacteria of the type injected; while the pathologic changes observed in sections stained with hematoxylin and eosin were in general those observed by others. While the changes varied in degree with the individual rabbit and the microorganisms injected; in general, they were a cholecystitis with some necrosis accompanied by desquamation of epithelium, capillary engorgement of red blood cells, perivascular infiltration and edema.

The results of the foregoing experiment naturally suggested many leads as to the probable cause of this loss of the pneumococcolytic activity of the bile.

1. In what percentage of human bile collected by gall bladder drainage is the pneumococcolytic action lacking?

2. Will the bacteria commonly found in cholecystitis such as the streptococcus, Staphylococcus aureus, Staphylococcus albus and B. coli communior, if suspended *in vitro* in normal rabbit bile for one hour, remove the pneumococcolytic activity?

3. Will the bacteria commonly found in cholecystitis growing in a bile medium destroy the pneumococcolytic activity of the bile?

4. Is the pneumococcolytic activity of bile removed by the presence of leucocytes (pus cells)?

Solution of each of the above questions was attempted by experiments which are outlined in detail in the order enumerated.

PNEUMOCOCCOLYTIC ACTIVITY OF HUMAN BILE

Twenty-four human bile specimens were collected by gall bladder drainage (Lyon's technic) under as sterile conditions as possible and cultured immediately. Within twenty-four hours the pneumococcolytic activity was tested as previously described. Of the twenty-four bile specimens tested, all possessed prompt pneumococcolytic activity. Thirteen of these specimens were apparently sterile on culture, and eleven showed growth of the bacteria commonly found in cultures of human bile. Whether these microorganisms were contained in the bile within the gall bladder or picked up in the insertion of the tube, it is not possible to conclude, although every precaution was taken to eliminate this possibility. The presence of the bacteria in the bile apparently did not interfere with the pneumococcolytic activity.

ATTEMPT TO REMOVE THE PNEUMOCOCCOLYTIC ACTIVITY BY SUSPENDING IN THE BILE THE BACTERIA COMMONLY FOUND IN CHOLECYSTITIS

The bile of three apparently normal rabbits was collected, mixed and found sterile on culture. The bile mixture was transparent and of an emerald green color. The pneumococcolytic activity of the bile mixture was determined as previously described. One c.c. of the bile mixture was then placed in each of four small sterile test tubes 12 x 80 mm.; this was followed by 0.2 c.c. of a two billion per cubic centimeter suspension of Streptococcus mitis in the first tube, Staphylococcus aureus in the second, Staphylococcus albus, and B. coli communior in the third and fourth tubes. The tubes were then placed in a water-bath at 38° C. for one hour, without apparent autolysis. The tubes were then centrifugalized to precipitate the suspended microorganisms and the clear supernatant of each tube removed to a second set of tubes. The clear supernatant of each tube was then tested for pneumococcolytic activity. In no instance, were pneumococci recovered, though a few colonies of the microorganism previously added were recovered. Table II gives in detail the procedure and results of this experiment and shows the persistence of the bacteriolysins of pneumococci in normal rabbit bile after attempts to remove them by the Streptococcus mitis, Staphylococcus aureus, Staphylococcus albus and B. coli communior.

TABLE II

TUBE CONTAINING 1 C.C. NORMAL BILE	TWO BILLION PER C.C. SUSPENSION PNEUMOCOCCI ADDED	WATER-BATH 38° C.	PNEUMOCOCCOLYTIC ACTIVITY	TWO BILLION PER C.C. BACTERIAL SUSPENSION ADDED	WATER-BATH 38° C.	CENTRIFUGALIZED	TWO BILLION PER C.C. PNEUMOCOCCUS SUSPENSION ADDED TO RECOVERED SUPERNATANT	WATER-BATH 38° C.	PNEUMOCOCCOLYTIC ACTIVITY
1	0.2 c.c.	1 hr.	Present	0.2 c.c. Streptococcus mitis	1 hr.	15 min.	0.2 c.c.	1 hr.	Present
2	0.2 c.c.	1 hr.	Present	0.22 c.c. Staphylococcus aureus	1 hr.	15 min.	0.2 c.c.	1 hr.	Present
3	0.2 c.c.	1 hr.	Present	0.2 c.c. Staphylococcus albus	1 hr.	15 min.	0.2 c.c.	1 hr.	Present
4	0.2 c.c.	1 hr.	Present	0.2 c.c. B. coli communior	1 hr.	15 min.	0.2 c.c.	1 hr.	Present

WILL THE BACTERIA COMMONLY FOUND IN CHOLECYSTITIS GROWING IN A BILE MEDIUM DESTROY THE PNEUMOCOCCOLYTIC ACTIVITY OF BILE?

The bile of five normal rabbits was collected and mixed and, on cultures, was sterile. A quantity of sterile hormone broth (P_H 7.8) was divided into two parts and to one part was added 5 per cent normal serum and 10 per cent of the rabbit bile. To the second part of the hormone broth, 5 per cent of

normal serum alone was added. Each of the lots were filled into test tubes in 10 c.c. amounts. The procedure was done in a sterile manner and on incubation for forty-eight hours at 38° C. the media remained sterile.

One tube of each lot was inoculated with the following microorganisms; Streptococcus mitis, Staphylococcus aureus, Staphylococcus albus, and B. coli communior. These cultures were incubated for forty-eight hours at 38° C. and an abundant growth of each microorganism developed. Uninoculated tubes of each media were incubated at the same time. The cultures were then centrifugalized and the clear supernatant removed. The pneumococcolytic activity of the supernatant fluid of each culture in the two media, was then determined with the following results:

1. The pneumococcolytic activity was present in the bile medium culture fluids of the Streptococcus mitis, Staphylococcus aureus, Staphylococcus albus and B. coli communior.

2. The pneumococcolytic activity was not present in the culture fluids of the same microorganisms in the medium without bile; colonies of pneumococci being quite numerous on the plate cultures, but not as numerous as found on the plate cultures of the uninoculated medium without bile control nor in proportion to the number of pneumococci added.

3. The uninoculated bile medium was highly pneumococcolytic, the turbidity produced by the suspended pneumococci disappearing within a few minutes after adding, while the uninoculated medium without bile had apparently no pneumococcolytic activity, numerous colonies of pneumococci appearing on the plate cultures.

THE EFFECT OF THE PRESENCE OF LEUCOCYTES ON THE PNEUMOCOCCOLYTIC ACTIVITY OF BILE

It has been suggested that possibly the leucocytes (pus cells) often found in cholecystitis were responsible for the loss of the pneumococcolytic action of the bile and that if a heavy suspension of leucocytes was added to normal rabbit bile they would affect this result. Accordingly the pneumococcolytic activity of 1 c.c. of sterile normal rabbit bile was determined as previously described and found to be present. A heavy suspension of washed rabbit leucocytes was prepared, and to 1 c.c. of rabbit bile 0.2 c.c. of the concentrated leucocyte suspension was added, the tube shaken and incubated in a water-bath at 38° C. for one hour. At the end of this time the pneumococcolytic activity of the bile was again determined and still found active.

DISCUSSION

It is appreciated that in the many reports in the literature on the bacteriology of human bile, the isolation of the pneumococcus is rare, but the fact remains that it has been isolated from a medium that has marked bacteriolytic action against it and it is to be regretted that no study was apparently made of the bile from which it was isolated, which, undoubtedly, would have given much valuable information. It is realized that the number of human bile specimens tested for pneumococcolytic activity was small; yet, this number may at least

be considered as a confirmation of the above reports and also as an index of the frequency of the existence of this condition.

It is evident from the preceding work that some change takes place within the gall bladders of rabbits infected with the Streptococcus mitis, Staphylococcus aureus, and Staphylococcus albus, which destroys the pneumococcolytic activity of the bile and delays this action in bile from gall bladders infected with B. coli communior for a period of three weeks. *In vivo* this work was confirmed in one rabbit infected with B. coli communior controlled by two normal rabbits.

The literature on the germicidal properties of hepatic duct and cystic bile for microorganisms other than the pneumococcus and streptococcus, is quite voluminous and in an excellent review given by Neilson and Meyer[5] one is quite impressed by the differences of opinion, four different properties being ascribed to bile *in vitro*, namely; it favors the growth of common bacteria, except pneumococci and streptococci; it is indifferent or it may be inhibitive, or it may even be germicidal. While no definite explanation was noted in the literature explaining the germicidal properties of normal bile for the pneumococcus, Avery and Cullen[6] attribute the solubility of the pneumococcus to enzymes contained within the organism, which, it is interesting to note, acts best at a P_H concentration most favorable to pneumococcus growth 7.0-7.8. While these factors apply to the bile of apparently normal and immunized animals, the extent of their application to bile from infected gall bladders of rabbits is limited; because from the findings here reported and other reports of bacteriologic examinations where pneumococci were noted, the germicidal and bacteriolytic effect on pneumococci was not present.

That the suspension of the Streptococcus mitis, Staphylococcus aureus, Staphylococcus albus, and B. coli communior in normal rabbit bile; the growth of the same microorganisms in a medium rich in bile; likewise, the suspension of leucocytes (pus cells) in normal bile did not remove the pneumococcolytic activity is suggestive that the ideal conditions exist only within the gall bladder or its walls, the infective microorganism producing there a pathologic condition interfering with the normal functions of the gall bladder; such as the concentration of the bile; conversion of the bile salts to other substances, and thus removing from the bile its germicidal and bacteriolytic power to act on pneumococci.

CONCLUSIONS

1. A bacterial cholecystitis was produced in rabbits by the direct injection of the Streptococcus mitis, Staphylococcus aureus, Staphylococcus albus, and B. coli communior into the gall bladder.

2. Bile from experimental bacterial cholecystitis of rabbits, produced by the above microorganisms except B. coli communior, loses its pneumococcolytic activity.

3. The pneumococcolytic activity of bile from cholecystitis of rabbits produced by B. coli communior, while present is somewhat delayed, and *in vivo* this result was confirmed.

4. Of twenty-four human bile specimens tested for pneumococcolytic activity, all were found to possess this characteristic.

5. Suspension of the Streptococcus mitis, Staphylococcus aureus and albus, B. coli communior, and leucocytes in normal rabbit bile did not remove the pneumococcolytic activity. Also cultures of these microorganisms in 10 per cent bile medium failed to remove this characteristic.

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A SIMPLE AND RAPID QUANTITATIVE TEST FOR ALBUMIN IN URINE*†

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INTRODUCTION

SINCE 1770, when its presence in urine was first demonstrated by Cotugno, the test for albumin has probably been performed oftener than any other chemical or clinical test. The deficiencies in our present knowledge of albumin excretion must, therefore, be laid to the lack of a satisfactory clinical quantitative method, because the statistical and clinical advantages of placing albumin determinations on a quantitative basis are, of course, self-evident. Particularly striking, at the present time, is the great contrast between the practical absence of data on albumin excretion and the vast accumulation of intensively worked out data on sugar excretion, which is daily increasing our knowledge of carbohydrate metabolism. These considerations, together with the experiences gained by typing albumin excretion by means of methods, shortly to be published,[1] led to the conclusion that a simple, accurate, and rapid method of measuring albumin in urine was something in the nature of a necessity.

Experimental work directed towards finding such a method was instituted by reinvestigation of the qualitative albumin tests in common use, because a test was aimed at from the outset which would require no more time or trouble for its performance than Heller's or the heat test which physicians for so long a time have been accustomed to employ. The possibilities of the

*From the laboratory of The Prudential Insurance Company of America, Newark, N. J.
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nitric acid ring and of the heat test for quantitative work were exhausted, however, without success, notwithstanding the many modifications which have been proposed for the purpose. The reasons why such failure is inevitable are easily understood when a method of attack such as ours is employed. This differed from those of other investigators in that from the beginning all experiments were made in duplicate with solutions of known protein concentration in water and urine. As regards Heller's nitric acid ring test, it may be noted in passing that no stratification, or layer, test can be made to yield reliable quantitative results with any technic whatsoever. Furthermore, the compositions of different specimens of urine influence the velocity of development and intensity of the rings which may form and so lead to false deductions. Thus it was observed that serum protein in distilled water solutions exhibited rings in concentrations of one part in 30,000 parts of water, but that the same concentrations in different samples of albumin-free urine often failed to show a ring in concentrations as low as one part in 12,000 parts of urine. With the heat test, it was found that a number of uncontrollable factors, physical and chemical, such as hydrion concentration, kind and amounts of salts in solution, intensity of heat applied, etc., affect the character of the coagula and prevent the achievement of uniform results. It is also worthy of mention that there are other variables, such as the presence of certain drugs and preservatives, which detract from the reliability of both Heller's and the heat tests.

Gravity tests, like Esbach's, which take hours to complete, or tests depending upon centrifugalization, were not considered within the scope of our work on account of previous findings of inaccuracy and because a test was wanted which would not only be free of the personal equation of the analyst, but which would also give prompt results with an irreducible minimum of manipulation and apparatus.

The precipitation characteristics of different protein precipitants were next studied, with the intention of selecting the one which promised to best fulfill the conditions necessary for measuring protein in urine as understood by us. In the order of their importance these are:

1. Specificity of precipitation for the proteins of present clinical interest in urine.
2. Quantitative precipitation.
3. Constancy of reproducibility.
4. Promptness of precipitation.
5. Sensitivity.
6. Suitability of precipitate for optical measurement.
7. Stability of reagent.
8. Simplicity of technic.
9. Elasticity of time element.
10. Economy.

The result of the trials favored sulphosalicylic, or salicyl-sulphonic acid, as it is sometimes called in the literature.

Older experimenters called attention to the fact that when sulphosalicylic acid is added to some urines an opalescence or cloudiness develops, which disappears upon warming, and they attributed such instances to the presence

of albumoses or peptones. In our own experience of 200,000 specimens tested with sulphosalicylic acid all such cases were found to be caused either by conjugates, by resinous substances, by protamines, or spermines, or similar large molecules associated with the presence of semen in urine. Cloudiness caused by substances other than albumin may be quite pronounced and easily mistaken for albuminous clouds, therefore, sulphosalicylic acid is specific for the proteins of clinical interest in urine only when used under certain definite conditions, to which I have called attention in previous papers.[2]

Every method of applying sulphosalicylic acid to urine should provide for warming tests which become cloudy. A little heat causes the cloudiness to disappear as the nonalbuminous substances go back into solution when warm. Albuminous clouds, on the contrary, when once established, remain unchanged, whether the test be cold, warm or boiled. They are easily distinguishable from the precipitate formed by Bence-Jones' protein with sulphosalicylic acid, because Bence-Jones' precipitates disappear upon boiling and are always very dense and appear exactly like curdled milk. The presence of Bence-Jones' protein should always be suspected whenever an albumin test yields an unusually dense precipitate of curdled appearance. When used under such conditions, sulphosalicylic acid may safely be regarded as being more specific for the proteins of clinical interest in urine than either Heller's or the heat test.

PRECIPITATION

The test to be described is grounded upon the albumin test previously described by me, which has been in routine use in the Prudential and other laboratories for years. Experience has confirmed its accuracy and reliability as a qualitative test, and satisfactory quantitative results have been uniformly obtainable with it in conjunction with the scopometer.[3] The test is empyrical but takes cognizance of the variables which arise in the treatment of urinary proteins with sulphosalicylic acid because the resulting precipitates are influenced by a number of factors. These factors include:

1. The concentration of sulphosalicylic acid.
2. The concentration of protein.
3. The relative dilutions of protein and acid.
4. The natures and proportions of different proteins (i.e., globulin-albumin).
5. Unknown substances in urine which may be adsorbed.
6. The kinds of salts present.
7. The quantities of salts present.
8. Temperature.
9. Time.

As regards concentration and dilution of precipitant and protein, it was assumed *a priori* that the employment of equal volumes of precipitant and specimen would make for the utmost technical simplicity and also greatly simplify any calculations which become necessary. It was therefore experimentally determined that the optimum concentration of sulphosalicylic acid for precipitating urinary proteins in this way was between 4 per cent and

7 per cent, and 5 per cent was adopted for the method as being ample for the precipitation of any possible concentration of albumin which might be encountered in urines and as being sufficiently acid to meet all likely reaction variables. Numerous specimens of different types of urine were then taken at random and tested with sulphosalicylic acid in three different ways: (*A*) equal parts of urine and 5 per cent sulphosalicylic acid; (*B*) one part of urine and twenty-four parts of 2 per cent sulphosalicylic acid; and (*C*) one part of urine and three parts of 3 per cent sulphosalicylic acid. Comparisons which were run on the same urine specimens with tests *A* and *B* showed that, when measured by comparable standards, 30 per cent to 40 per cent of the various specimens exhibited very considerable discrepancies in the protein values obtained with *A* and *B* tests. It was also the rule in every case in which a discrepancy was found that the *B* test gave higher results than the *A* test. When the protein was isolated from such urines, and estimated gravimetrically, or by nitrogen determinations, the checks, with a single exception, were closer to the *A* test, made with equal parts of urine and reagent. It was also found in every instance in which there was such a discrepancy that further dilution would magnify it. Runs were also made on the same specimens, using equal parts of 5 per cent sulphosalicylic acid and urine (*A*), and one part of urine to three parts of 3 per cent sulphosalicylic acid (*C*). As was to be expected, the results showed the discrepancies to be fewer and less than when one part of urine was treated with twenty-four parts of 2 per cent sulphosalicylic acid (*B*).

Here, again, however, when the discrepancies were checked by nitrogen

TABLE I

ONE SERIES OF DISCREPANCIES FOUND BY TREATING URINES WITH SULPHOSALICYLIC ACID IN THREE DIFFERENT WAYS

DISCREPANCY NUMBERS	MILLIGRAMS OF ALBUMIN PER 100 C.C. URINE				NOTES
	A	B	C	GRAVIMETRIC AND KJELDAHL'S CHECKS	
1	75	200	–	72	A—2 c.c. urine: 2 c.c. sulphosalicylic acid 5 per cent
2	30	75	–	25	
3	50	100	–	55	B—1 c.c. urine: 24 c.c. sulphosalicylic acid 2 per cent
4	25	75	–	21	
5	50	150	–	55	C—2.5 c.c. urine: 7.5 c.c. sulphosalicylic acid 3 per cent
6	20	200	–	31	
7	40	200	–	50	Specimens, nos. 1 to 10 and 16 and 17, were checked by the gravimetric method. The urines were adjusted to P_H 4.6-4.8, heated in boiling water, and centrifuged. The coagula were then washed and weighed
8	160	300	–	170	
9	40	100	–	50	
10	50	100	–	64	
11	50	100	–	62	
12	80	100	–	92	
13	120	200	–	120	Nos. 11-15 were checked by Kjeldahl's method. The albumin was isolated in the same way as in the gravimetric checks, and nitrogen determinations made (Nx 6.4).
14	300	1000	–	336	
15	125	200	–	113	
16	135	180	150	105	
17	60	125	100	77	Specimen No. 15 when diluted 1:74, and *B* test again applied, read 1000 mg. per 100 c.c.

determinations the results were always closer to the values obtained by testing with equal parts of precipitant and urine.

These results could not be taken as meaning otherwise than that the relative dilution and concentration of urine and precipitant greatly influence the optical effects by which estimations are made, and that the employment of equal volumes of urine and precipitant not only offered by far the simplest technic but also led to greater quantitative accuracy than any other method of precipitating protein in urine with sulphosalicylic acid.

If the same solution of sulphosalicylic acid be added to similar concentrations of albuminous urine and aqueous solutions of serum protein, it will be observed that the precipitates show differences. The urinary precipitates appear to be finer and in better equilibrium, as evidenced by the fact that they stay appreciably longer in suspension before flocculating. This offers at least a partial explanation of the failure of nephelometry and turbidimetry to yield consistent checks with freshly prepared protein standards. In urine the difference suggests that the effects may be explained by its higher viscosity with greater internal friction and the presence of something like protective colloids. Probably both of these combine with salt effects. In order to eliminate, if possible, the variables encountered in urine so as to get as uniform precipitation as possible, attempts were made to modify the character of the sulphosalicylic-protein precipitate. These demonstrated that the presence of sodium sulphate modifies the character of the precipitate obtained with albuminous urine and aqueous solutions of protein in such a way as to make them more alike. Sodium sulphate was therefore added to sulphosalicylic acid in reagent form.

ESTIMATION

The next step was directed towards finding the easiest possible method of measuring the precipitates or turbid suspensions developed with the reagent and albuminous urine. No standard of measurement which did not promise to be stable or permanent was considered. Originally it was thought that some kind of cloudy glass in the form of tubes or a wedge could be found or made to answer the purpose in conjunction with some sort of contrivance for gauging it against the cloudiness of the urine tests. When it became necessary, however, to confess the futility of thorough search and efforts along these lines, a different angle of approach was sought and numerous attempts were then made to find or concoct some sort of emulsion, or sol, or inorganic precipitate—in fact, anything which might work as a satisfactory standard for measuring turbidities.

When this line of attack presented no solution the possibilities of gels like silicic acid and gelatine were next exploited, and Mr. Clyde Brockett, of the research laboratory of the Eastman Kodak Co., succeeded in making a series of standard tubes which matched beautifully with the urine tests. These were shown at the San Francisco meeting of the Society and proved satisfactory in every other way but permanency. The gels liquefied sooner or later and settling of the particles spoiled the tubes.

With experience it became plain, practically as well as theoretically, that any tubes intended for comparison standards must be made under exactly

the same conditions as the protein in urine is precipitated, or under the closest approximate conditions which could possibly be contrived. A return was therefore made to our earliest experiments in preparing the fresh standards for nephelometry, because a possibility of finding some way of manipulating these into a permanent condition was still open. Clear, albumin-free urine was boiled and series of standard tubes were made by adding to each a known concentration of protein and precipitating in precisely the same way as in making the urine tests, and sealing the tubes immediately. These standards also proved satisfactory in every way except permanency. They were easy to reproduce and some sets made in this way remained serviceable for months. Sooner or later, however, changes took place in the urine which spoiled the tubes.

Trials were then made in the same way with aqueous solutions of different proteins, and what was considered a partial success was thus achieved. Some sets of these tubes kept without apparent change for six months or more and were used under careful control in the Prudential laboratory and exhibited at meetings as long ago as 1918. The changes in the aqueous tubes were of a different order from those which took place in the urine standards. The change in the urine tubes was striking. They would suddenly darken and become very turbid—generally overnight. In the aqueous tubes, on the other hand, deterioration took place slowly and gradually, and the only kind of changes which it was possible to detect in them affected the condition of the precipitates. The particles would either lump, so as to take on a granular appearance, or by a distinctly different form of coalescence the particles would agglomerate in shreds. It was often possible to break up the agglomerates by violent agitation and thus prolong the usefulness of the tubes.

It was found practicable to reproduce such tubes easily and surely, and as no changes were perceptible other than the coalescence or agglomeration of the particles, our problem finally seemed to narrow itself down to contriving some means of preventing the eventual coalescence or agglomeration of the particles. The effects of substances which raise viscosity and internal friction, and of protective colloids, were therefore studied because they seem to be present in urine. At the same time experiments were also made with a number of dyes which were known to be adsorbed by protein. Of these, the indicator dye, bromphenol blue, was tried, at the suggestion of Dr. H. Sharlit, of the Harriman Research Laboratory, because it has a yellow color in acid solutions which is not unlike that of normal urine. Bromphenol blue apparently stabilizes the sulphosalicylic-protein precipitate by being adsorbed on the particles, which take on its yellow color. This effect increases the visibility of the particles or cloudiness and makes the test more sensitive. The dye also acts to diminish the color contrasts between urine and standard tubes, and gives the additional advantage of eliminating opalescence, which is sometimes bothersome in comparing undyed turbidities.

Starch and gum arabic apparently are not affected by sulphosalicylic acid, and when solutions of serum protein are made with either of these, the resulting precipitate has every appearance of being like that of albuminous urine of the same concentration which has been treated with the reagent con-

taining the dye. By including gum arabic in the serum solutions, and by precipitating with a reagent containing bromphenol blue, sets of standard tubes have been made which have kept for well over a year without perceptible change in color or difference in cloudiness values. These have been considered to meet the permanency requirement.

ALBUMINIMETRY

It has been customary to compare samples of cloudy liquids by either holding them up to the light or by viewing them against a black background. Individual preferences usually decide the matter, although it is generally accepted that the denser dispersoids are best compared by transmitted light, and the finer turbidities against a black background. In watching the work of the technicians in the Prudential laboratory, it was noted that difficulties arose whenever it was sought to have all of them make turbidity comparisons in the same way. Each one seemed to favor some particular spot in the laboratory where he thought that the lighting was best adapted for distinguishing differences in opacity. Some technicians preferred to compare three tubes instead of two by placing the unknown sample between the two standards nearest to it, viz., the next denser and the next lighter. Undoubtedly such practice tends to hasten decision and increase the delicacy and accuracy of reading. Some workers, as is well known, experience more difficulty than others in making turbidity comparisons and manifest indecision by trying out more standards and by trying them oftener and under more diverse lighting conditions and against various backgrounds. In the effort to improve and make more uniform matters of technic, it was learned that inaccuracy and indecision were usually traceable to one or more of three different causes:

1. Inequality of lighting the tubes.
2. Reflections between the tubes.
3. Differences in the colors of standard tubes and urine tests due to the variable colors of urine.

There are other factors which probably play a part in causing hesitation at times, but experience has shown that when those which have been mentioned are eliminated, even novices are able to make correct readings quickly and without indecision.

As some sort of an arrangement was necessary for conveniently keeping and handling the standard tubes, it was decided to design a rack and incorporate in it a simple device for albuminimetry which would prevent reflection between the tubes and provide for equality of lighting them. (See Fig. 1.) In addition to these, an opacitimetric gauge was proved experimentally and found to facilitate and standardize readings and has been provided by interposing a broad, black line on a white background behind the tubes. When light falls on the tubes and passes through the turbid media in them, the visibility and shading of the black line show changes which are directly proportional to the opacity of the media through which light reaches the black opacitimetric line. Very definite turbidity comparisons are thus made practicable, and the method is sensitive to a degree which allows the use of standard tubes approximating each other so closely that similar readings would be otherwise

impossible without the aid of a complicated and expensive optical instrument. This clinical albuminimeter permits one to hold the tubes up to the light (nephelometry), if one prefers to match that way; or, by an instantaneous shift, to mark the shading of the black line back of the tubes by having the light fall over the shoulder of the observer; or, better yet, by having the light from overhead fall on the tubes, held at an angle in front of a light background, such as a towel or piece of paper laid on the table (opacitimetry). Worked in the lower ranges, this device will register differences as minute as those represented by one part albumin in 50,000 parts of urine without difficulty in either artificial or sunlight.

It goes without saying that the suspensions in the standard tubes should always be made homogeneous before matching, by turning the tubes. If a practice be made of keeping each tube in its proper order, or pocket, in the

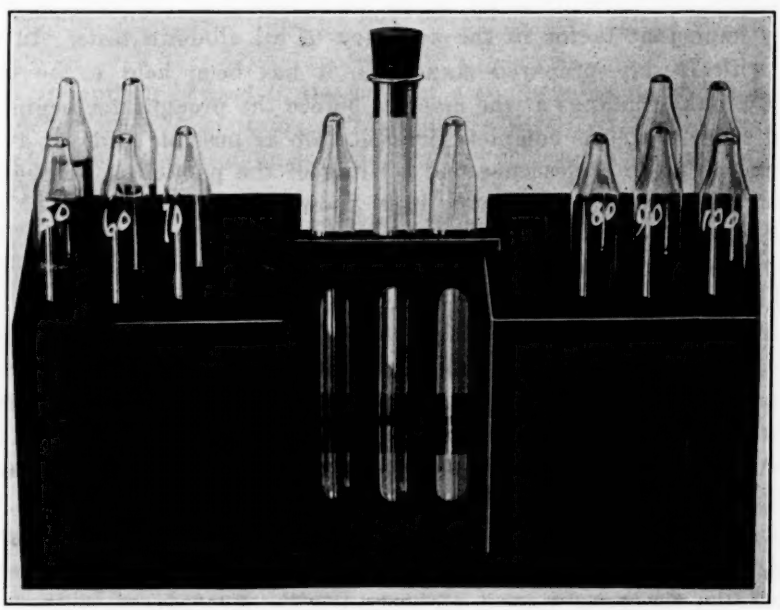

Fig. 1.—Exton's clinical albuminimeter. In order to show method of reading opacities the light filter has been removed.

rack when handling them, a little experience will lead to automatically picking the right tube and replacing it in the right pocket. For convenience when making several tests, empty pockets are provided for a few extra tubes.

Examination with the spectroscope shows that urochrome, or the coloring matter of normal urine, absorbs blue light; darker urine also absorbs part of the green light of the spectrum, and the absorption of the bromphenol blue in acids as used in the reagent is similar, therefore, if the urine tests and standard tubes are viewed through a light filter which cuts out the light that would be partially absorbed by urine and which lets through only the light which is unaffected by the color of the urine, differences in color between standard tubes and urine tests disappear. Yellow and red light filters of selected spectral transmission are available which will satisfy these requirements, and are conveniently and with advantage combined with the other fea-

tures of the clinical albuminimeter. This simple expedient is efficient in eliminating practically all of the difficulties encountered in comparing turbidities which arise from differences in color, and a trial of the experiment will demonstrate that the differences in color between water and urine and bromphenol blue reagent are practically annihilated when samples of these are viewed, side by side, at the same time, through a filter of proper spectral transmission. The combination of comparing three tubes with equal lighting and without reflections, with opacitimetric black line and light filter, avoids every difficulty which has arisen within our experience in measuring cloudiness, and, as embodied in the clinical albuminimeter,* affords an unexpectedly easy, certain, precise, and sensitive means of comparing and measuring opacities.

TIME ELEMENT

The time after precipitation when the opacities or suspensions are read is a very important factor in the accuracy of all albumin tests. In turbidimetry, with freshly prepared standards, it has been held to be the best practice to make readings at the moment before the precipitates begin to flocculate in order to get as complete precipitation as possible and yet avoid the interferences due to coalescence and settling of the particles. In the case of sulphosalicylic-protein precipitates, it is known that the velocity of precipitation depends upon the concentration of protein in the specimen. The correct timing of an unknown concentration of protein is, therefore, a practical impossibility. To try to meet this difficulty by fixing the same arbitrary time for reading all concentrations is a makeshift which becomes very troublesome because it obligates the analyst to mark time by the clock in order to make his comparisons or readings at a certain definite instant, before or after which readings will give inconsistent results. This method of timing also has the objection that it precludes warming the tests, which sacrifices the specificity for albumin,—perhaps the greatest advantage of sulphosalicylic acid.

It was possible to study some phases of precipitation and behavior of particles in suspension with the scopometer, and by this means observations were made on the precipitation of protein with our reagent, with reference to time and reducibility. As to time, it was learned that precipitation goes on faster as protein concentration increases. As to reproducibility, it was found that if precipitation be allowed to go on beyond the flocculation point, homogeneous suspensions could again be secured in all concentrations by simply turning the tubes upside down, and repeated scopometric measurements of the turbidities produced by different concentrations of protein established the reproducibility of such suspensions even after the precipitates had been settled in the bottoms of the test tubes for hours.

These results pointed to the practicability of applying heat in order to accelerate precipitation and make the test specific for albumin. They also showed a way to rid the test of the troublesome time element with its burden of clock watching. By having the values of the standard tubes represent complete precipitation of the protein in them, it is only necessary to let the urine

*Standard tubes, reagent, and rack may now be obtained from Lehn and Fink Inc., of New York City.

tests stand until precipitation is practically complete. They can then be read against the standards at any convenient time thereafter.

ACCURACY

Under the usual conditions of clinical work the experimental error of the method should be within five mg., or one part albumin in 20,000 parts of water. This degree of accuracy is attainable by waiting five minutes, or longer, before warming and reading the test. If haste be not necessary it is handier to let the tests stand until some later time when it is convenient to read the results. If greater accuracy be desired, dilute the specimen so that the concentration of protein falls within the range of 20 mg. to 50 mg. per 100 c.c., and let precipitation go on in an incubator for several hours. Then read the homogeneous suspension against intermediate standard tubes representing 5 mg. difference in value. By careful work in this way results are possible within an experimental error of 2 mg., or one part albumin in 50,000 parts water.

REAGENT

The precipitant is used in the form of a single solution, which appears to keep indefinitely. It contains sulphosalicylic acid (Eastman), sodium sulphate (crystals), and bromphenol blue (clear, saturated, 0.4 per cent watery solution).

To prepare: Dissolve 50 grams of sulphosalicylic acid* and 10 grams of sodium sulphate in about 800 c.c. of distilled water, and add 25 c.c. of the dye. Make up to one liter and filter through acid-washed paper (Whatman No. 40).

THE STANDARD TUBES

Convenience is gained by limiting the number of standard tubes to an adequate minimum, and every clinical requirement is met by having them differ from one another by 10 mg. of albumin, or one part in 1,000. For closer work it is just as easy to make intermediate tubes and read them in the clinical albuminimeter. The sets of standards used in the Prudential laboratory for routine work range from a blank to 100 mg. of albumin, so that they comprise a series of twelve tubes, representing 0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg. protein per 100 c.c. We have adopted the mille per cent expression for values because in practice it has proved simpler, safer, fitter, and more convenient than any other. Urine which reads more than 100 mg. is diluted with water, either before or after precipitation. Urine which reads between the blank and 5 mg. or 10 mg. tubes, can be read satisfactorily, because error in the low ranges is greatly diminished and clinically negligible. Some may find it easier, however, to increase the proportion of urine to reagent, and read against standards of higher values.

Preparation of the tubes is simple but demands careful work for precise reproduction.

It is very essential to work with scrupulously clean apparatus and to take every possible precaution to exclude foreign matter like dust, etc.

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*Samples of sulphosalicylic acid found in the market vary greatly in purity and color of solutions. The addition of concentrated sulphuric acid (no more than needed) will often cause the pink color to disappear.

I am glad to acknowledge here a debt of thanks due C. E. K. Mees and H. T. Clarke, of the Eastman Kodak Research Laboratory, for improving and refining their product. Five per cent solutions made with it are perfectly free of color if filtered through acid-washed paper.

TABLE II

REPRODUCIBILITY OF STANDARD TUBES MADE FROM BLOOD OF DIFFERENT ANIMALS AND AT DIFFERENT TIMES

I	F	E	D	M	N	O	P	NN	R	S
100	100	100	90	100	100	100	100	100	100	100
80	80	80	70	80	80	80	80	80	80	80
60	60	—	—	60	60	60	60	60	60	60
50	50	50	45	50	50	50	50	50	50	50
40	40	40	—	40	40	40	40	40	40	40
30	30	—	35	30	30	30	30	30	30	30
25	25	25	25	25	25	25	25	25	25	25
20	20	20	20	20	20	20	20	20	20	20
15	15	—	—	15	15	15	15	15	15	15
10	10	12	9	10	10	10	10	10	10	10
5	5	5	—	—	5	—	5	—	5	5

I—Sheep serum.
F—Sheep serum.
E—Sheep serum diluted with normal urine.
D—Pig serum.
M—Steer plasma.
N—Steer plasma diluted with 1 per cent NaCl to the 100 mg. protein concentration.
O—Steer serum.
P—Sheep serum.
NN—Sheep serum diluted with gum arabic solution.
R—Sheep plasma.
S—Sheep serum from same animal as R.

The serum, preferably sheep, must be as fresh and free from hemoglobin as possible, because we have a very distinct impression that old serum, even when kept in a chilled condition, does not make as satisfactory tubes as does fresh serum. We have found it best to collect the blood in clean, shallow pans, and let it clot well before decanting the serum into wide-mouth containers. The clear serum is promptly and thoroughly centrifuged (more than once at high speed), and samples are taken for protein estimations by Kjeldahl nitrogen methods (Nx6.4), or more conveniently by diluting and precipitating with the reagent and reading the scopometer scale. The serum is then diluted with 5 volumes of 2 per cent NaCl solution. Further dilutions are made with distilled water on the basis of the known protein content so as to get a stock solution containing 200 mg. protein per 100 c.c. One per cent and 0.5 per cent solutions of purest, finely ground gum arabic (i.e., clear) should be ready at hand. The desired series of protein concentrations is now obtained by diluting portions of the 200 mg. stock with distilled water and the gum arabic solutions. The proportions of gum arabic and water should be such as to make a 0.5 per cent solution of gum arabic in each protein tube.

We use 125 mm. by 12.5 mm. clear glass stock test tubes. They are calibrated by selecting those in which equal volumes of water stand at the same level, and cleaned with bichromate-sulphuric acid mixture. They are then washed several times in distilled water and dried in the oven. About fifteen tubes are needed for a set and these are constricted about one inch from the mouth by drawing them out in the flame. This helps to expedite neat sealing. Small funnels (Fig. 2) are also prepared from the test tubes by drawing them out in the flame to a diameter that will go through the constrictions of the tubes. These facilitate filling and prevent wetting the constrictions.

Four c.c. of the desired protein concentrations are then poured into the tubes through the funnel, and filling is completed by adding 4 c.c. of the reagent. The tubes are sealed immediately. This is easily done by tapping

off the tops of the tubes at a file scratch on the constrictions and slowly revolving the open points in the flame of a blast lamp.

When cool, gently invert the tubes, to insure complete mixing, and be sure to turn the tubes gently several times a day during the first ten days. Diamond ink answers for marking the tubes. It seems scarcely necessary to caution against letting the tubes lie lengthwise, or stand upside down, and, of course, exposure to extremes of temperatures should also be avoided.

An occasional set of tubes will turn out less perfectly than others, due to unexplained differences in sera. Such anomalies become apparent in forty-eight hours. Sets of tubes made at bimonthly intervals over a period of eighteen months have been carefully and frequently compared with reference to optical values and the condition of the suspensions, and have been found to check perfectly and keep without apparent change.

The matter of color permanency was watched with special interest because it was feared that the dye would fade. No changes, however, have been

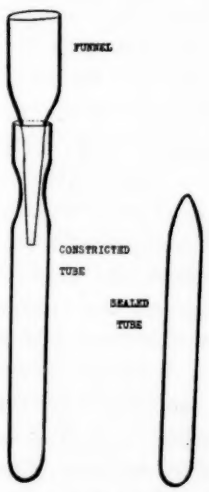

Fig. 2.—Glass parts used in making the sealed standard tubes.

detected. In this connection it may be of interest to note that we subsequently and accidentally learned that dyers make empirical use of the fact that dyes which show absorption in the ultraviolet region of the spectrum are made fast by mixing with them some salt having a similar absorption. Bromphenol blue and sodium sulphate show absorption in the ultraviolet.

In the course of the work it was necessary to make hundreds of experimental tubes, and I am happy to record my appreciation of the careful assistance given throughout by John Huizer, who is also entitled to credit for the technic of filling and sealing the tubes. I desire also to gratefully acknowledge the very material help in many ways of Anton R. Rose, who, with the assistance of Fred Schattner, thoroughly checked our earlier experiments.

DIRECTIONS

The test is calculated so that the cloudiness or opacity which results from mixing equal parts of urine and reagent denotes the number of milligrams of

albumin in 100 c.c. of urine. Clean test and standard tubes are essential for making the test, and cloudy urine should be cleared, if possible, by filtering, or by centrifuging and testing the supernatant urine. *Add 2 to 3 c.c. of reagent to a like amount of urine and let stand five minutes, or until it is convenient to read the result with the clinical albuminimeter.* Turn the tubes gently to make the suspensions homogeneous, and warm the test immediately before reading by passing the tube in and out of the flame several times in order to avoid boiling which is undesirable except when differentiating Bence-Jones' protein. The absence of cloudiness denotes the absence of albumin in the urine, while the presence of cloudiness means that the urine contains albumin, which is then measured with the albuminimeter. *Turn the albuminimeter rack (cover on) to have the suspensions in the standard tubes homogeneous.* With the test in the middle one of the three pockets, try out the standard tubes by placing them in the pockets on both sides of the test. Comparisons of opacity or cloudiness will show which two standard tubes closest approximate the test, i.e., a denser and a lighter one. *The result is then read by a match or by taking the reading between the two tubes next higher and next lower than the test.* If the test be cloudier than the 100 mg. standard tube, dilute five or ten times with water and multiply the reading by the number of dilutions; for example, if the original test consists of 4 c.c. (i.e., 2 c.c. urine and 2 c.c. reagent), dilute with water to 20 c.c. and multiply the result by 5. Some may find it more convenient to make a fresh test and dilute the urine with water before precipitating. *To make the test on cloudy urine* which cannot be cleared, dilute a portion of the urine with an equal volume of water and read against the standard tubes; precipitate a similar portion of the urine in the regular way and read against the standard tubes; the difference between the two readings measures the protein content. Urine containing large amounts of carbonates or other alkaline salts is apt to froth when any albumin test is applied, and it is better to acidify such urine with acetic or some other acid before testing. Decomposed or extremely alkaline urine will turn blue or purple when the reagent is added to it because bromphenol blue is also an indicator on the alkaline side. Such urine should also be acidified before making the test. If the color of a specimen should happen to be very dark or unusual, dilution and the light filter will bring it within the color range of the standard tubes.*

CONCLUSIONS

A simple and rapid quantitative test for albumin in urine has been described. Every contingency which is known to arise in the examination of urine for albumin is met by the method, which requires no more time or manipulation for its performance than do the qualitative clinical tests in common use.

The specific and quantitative features of the new test impart an accuracy and uniformity to albumin determinations which make them clinically valuable to a degree hitherto impossible.

*If a correct light filter is not available make a color control solution containing the same amounts of dye and sodium sulphate as the reagent and 5 c.c. of concentrated sulphuric acid to the liter. The control solution has the same color as the reagent and avoids the need of a light filter if substituted for distilled water when diluting the urine.

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ON THE INFLUENCE OF ACIDS UPON THE VIABILITY OF BACTERIA*

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INTRODUCTION

IT has already been clearly settled by numerous researches of preceding authors that the reaction of media has great influence upon the viability of bacteria.

The effect of reaction of media upon B. coli communis, which I employed in my experiment, has been repeatedly studied by many workers; their most important results are given in the table on page 736.

The results may be summarized thus: The width of the reaction allowing viability of B. coli lies between P_H 4.6-9.0, the optimum being in a rather weak acidity of the media.

The minute differences among the results of these authors may be probably ascribed mostly to the individuality of the strains employed in their experiments, and furthermore the methods of cultivation and composition of media as well as the temperature of incubating may have played some rôle on the different attitude of the organisms.

The methods, upon which these authors determined the death-point of bacteria, may be divided into two groups:

1. Determination of the death-point by estimating the acidity which the bacteria produced after development on media with sugars.

2. Determination of the death-point by observing the occurrence of growth in media, to which acids or buffer solution have been added. In the latter case the acidity or alkalinity of the media has been conveniently defined by the addition of HCl, or NaOH as the case may be, to the media.

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		AUTHORS	P_H (DEATH-POINT)	P_H (OPTIMUM)	MEDIA	BIBLIOGRAPHY
1	1912	Michaelis and Marcora	5.0		Lactose-Broth	Z. Imm. u. ex. Therap. I Teil, (orig.) xiv, 170.
2	1917	Shohl & Janney	4.6-9.6	6.0-7.0	Urine	J. Urology, i, p. 211.
3	1917	Clark & Lubs	4.5-9.0	6.0-7.0	Broth	Jour. Bact., ii, 1.
4	1918	Wyeth	4.3		Broth	Biochem. Z., xii, p. 382.
5	1919	Cohen & Clark	4.5-9.0	6.0-7.0	Broth	Jour. Bact., iv, p. 409.
6	1920	Chambers	4.9		Glucose-Broth	Ann. Missouri Botan. Garden, vii, p. 249.
7	1921	Dernby	4.4-7.8	6.0-7.0	Autolysated broth	Ann. Inst. Past. T., xxxv, p. 277.
8	1921	Scheer	4.7		Broth	Z. Imm., xxxiii, p. 36.
9	1922	Adam		6.4-7.1	Broth with and without sugar	Centralbl. Bakt., i, Abt. Bd., lxxxvii, p. 450.
10	1923	Dernby & Näslund	4.5-9.0	6.0-7.0	Broth	Z. Imm., I Teil, xxxv, Heft 5-6, p. 450.
11	1923	Kojima	4.8		Broth with sugar	Japan J. Exp. Med., vii, p. 1029.

It is to be regretted that few have paid much attention to the kinds of alkali and acid to give the media the desired reaction, i.e., hydrogen-ion concentration. Nor can I judge from my own experiment whether it is right or not to determine the acidity only by the actual acidity. A most complicated relationship of the acids and the growth of bacteria should be expected owing to the difference of acids, i.e. anion, dissociation-constant and valency etc., which must give widely diverging effects to the bacteria according to the kinds of chemicals employed.

Kojima[1] reported that the end-reaction, namely the death-point of bacteria of media, varies very much, according to the kinds of sugar added to the culture fluid. This resulted presumably from the different products in these media. Also the difference, qualitative as well as quantitative, of the organic acids contributed to the variation of the results.[2, 3]

On the other hand Krönig and Paul,[4] 1897, gave some notes on the bactericidal action of acids in their report, and described that bacteria are affected not only by the cation, i.e., H, but also by the anion and undissociated molecules. Other authors, however, differed in their method of examination and materials in their experiments, or employed entirely different acids; accordingly their results very often show no agreement.

For instance, Bial[5] found in 1902 that the glucose fermenting power of yeast is affected by organic as well as inorganic acids, and especially that the greater the dissociation the more the fermentation is interfered with. In such a minute concentration of the acid, as he describes, the action of the anion is scarcely noticeable and so the inhibiting action must be considered totally due to the dissociated H.

In 1906, Winslow and Lochridge[6] reported that "The mineral acids, HCl and H_2SO_4, are fatal in concentrations at which they are highly dissociated. Their action runs parallel, not to their normal strength, but to the number of free hydrogen ions per unit volume.

"The organic acids, acetic and benzoic, are fatal to the typhoid and colon bacilli at a strength at which they are only slightly dissociated. The effect here appears to be due to the whole molecule and is specific for each acid, acetic having only 10-20 per cent the toxicity of benzoic."

Johannessohn,[7] in 1912, published another report, in which he made similar experiments as Bial with a series of formic acid, and came to the conclusion, that with acids the main part of the acid action is played by the undissociated molecules, not by the ions; and besides the larger the molecular weight the more pronounced is the bactericidal action.

Michaelis,[8] however, gave an account on the same subject in 1914, and agreed with Bial, saying: "Da sich herausgestellt hat, dass alle chemischen und biologischen Vorgänge, die von der Acidität der Lösung beeinflusst werden, *nur die aktuelle Acidität* (d.h. die durch die Wasserstoffzahl definierte Acidität) massgebend ist, so erhellt die überwiegende Bedeutung derselben."

Against the results obtained by Michaelis, Traube,[9] 1921, published another opinion. He pointed out that some of the measures employed by Michaelis should be discarded and concluded that the acid action must be reduced rather to anion and undissociated molecules than to hydrogen-ion concentration, and further in biologic respects he concluded as follows: "Nach meiner Ansicht ist es viel richtiger, wieder die alte Titrationsacidität und Titrationsalkalität mehr in den Vordergrund der biologischen Betrachtungen zu stellen."

From physicochemical points of view, some authors attempt to compare the relationship between acid action and bacteria with surface tension, absorption, swelling and flocking character, osmotic action and electric potential of materials to be brought in contact,[10, 11, 12] but these can give no satisfactory explanation of the complicated processes in this problem. Such reactions as the coagulation of casein and swelling of fibrin, can at any rate, be taken as phenomena occurring in bacteria, which are composed at least of protein substances numbering 2,432, 902,008, 176,640,000 components.[13]

Furthermore, if other circumstances such as utilization of various organic acids by bacteria as the source of carbon are taken into account,[14, 15] it may be reasonable to think that at the same optimum hydrogen-ion concentration of the solution, some different results may be obtained according to the kinds of acid taken in adjusting the reaction of culture media. This fact must especially be taken into account, inasmuch as the death-point of bacteria, i.e., the so-called physiologic constant is mostly subject to the chemicals employed, and decided differences may result from the composition of the fluid.

These are the cardinal points, which have led me to attempt the following experiments.

EXPERIMENTAL

I used a strain of B. coli (C_{26}), which was employed throughout the entire course of my experiments. The standard culture media were peptone (Witte) solutions, thus prepared: The peptone was dissolved in aqua distillata to 1.0 per cent. No salt was added to the media.

Any correction of reaction was never attempted, because this peptone (Witte) solution showed P_H 6.8 just after preparation, and after one hour's sterilization at 100° C. invariably gave P_H 7.2, which made any correction superfluous. Further, any addition of acid or alkali would cause great inconvenience in the succeeding experiments.

The media which were used in stock cultures and in counting of the number of colonies were all routine agar and were adjusted with HCl and NaOH to the initial reaction of P_H 7.0.

The number of bacteria to be implanted at first need not be absolutely accurate,[16] in fact it is almost impossible to secure any constant number of bacteria in such experiments. However, I attempted to obtain the greatest possible exactness in the number of bacteria by the following method:

A twenty-four hour culture on agar medium is transferred into the standard peptone solution. After three generations in this medium (three days after) a platinum needle, which is provided with a mark 1 cm. from the point, is dipped in the fluid culture. This 1 cm. portion of the needle conveys about 5,000 bacteria to the next tube.

Test tubes containing the fluid media thus implanted, are incubated at 37° C. After the lapse of various periods each 1.0 c.c. fluid is taken off the tube and sown on agar plate, for the further cultivation and counting of the number of colonies.

Here the serial dilutions of culture fluid were performed with the utmost care, each dilution being prepared in secondary tubes from the original, 1:100, or if necessary with 1:100,000 solutions. The usual decimal dilutions with one and the same pipette must be avoided in order to obtain relatively constant results.

The estimation of hydrogen-ion concentration was performed throughout the examinations according to the electrometric method with outfits arranged after Clark.

As to the operation of the apparatus, I am much obliged to Dr. Kojima for his helpful advice and kind assistance.

EXPERIMENT I

As is shown in Table I the reaction of the media displays a transitional change to acidity after twelve to eighteen hours, when a most vigorous increase may be observed.

However, after twenty-four to thirty-six hours, it gradually returns again to the initial point and even to higher alkalinity. As to the transitional changes into acidity, some authors found the same result with broth culture without addition of sugar, and they attempted to ascribe the cause to the minute contents in the original broth. In my case no sugar could be detected in peptone solution, which may be concluded from the following tests.

1. The addition of Fehling's reagent to the boiled peptone solution always gave a negative result.

2. The same test was made with peptone solutions, which had pre-

TABLE I

THE DEVELOPMENT OF B. COLI IN 1.0 PER CENT PEPTONE SOLUTION AND THE CHANGES OF HYDROGEN-ION CONCENTRATION

INCUBATION TIME IN HOURS	NUMBER OF BACTERIA PER C.C.	P_H
0	520	7.21
6	135,000	7.21
12	10,500,000	7.14
18	54,000,000	7.07
24	83,420,000	7.10
36	130,200,000	7.22
48	137,700,000	7.35
72	98,000,000	7.41

viously been hydrolyzed by boiling in weak acidity after addition of HCl and thereafter reduced with NaOH to the neutral point.

Any other sugar test together with the former always gave negative results; no reducing substances were proved in the fluid; so it is incorrect to say, that the transitional increase in acidity is due to the minute sugar content. It is possible that the proteose and amino-acids in the course of hydrolysis or oxidation by bacteria may give acidifying substance to the media, and this may probably be the cause of the primary changes in reaction.

EXPERIMENT II

I examined here, as given in Tables II to XIV, the death-point of B. coli with thirteen different inorganic and organic acids. The peptone solution was prepared in 2.0 per cent watery solution, and increasing quantities of one-fifth normal acid were added to each 50 c.c. peptone water. All the mixtures received in addition, a certain amount of distilled water to attain 10 c.c. The initial reactions of these solutions are given in P_H after one hour's sterilization at 100° C. As the culture fluids here employed contain only a minimal amount of substance to show buffer action, they are liable to undergo remarkable changes during the development of bacteria, so the changes of P_H were given in every stage as often as possible during the course of incubation. The death-point in these cases should be determined relative to the initial reaction of the fluids.

TABLE II

HYDROCHLORIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	x ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	4.5	4.37	272		81	4.43	0	
2	4.2	4.51	660		64	4.51	57	
3	3.7	4.65	1,080		1,840	4.65	33,320	4.70
4	3.4	4.82	1,300		2,000		770,000	4.90
5	3.2	4.89			3,000		25,200,000	
6	2.8	6.01	1,100		3,200		28,210,000	5.10
7	2.5	5.41	1,800		985,000		29,200,000	5.53
8	2.2	5.57	5,000		2,100,000	5.75	49,200,000	6.10
9	1.8	5.98	11,000		2,700,000	6.38	78,000,000	6.73

x indicates in every case $\frac{N}{5}$ acid volume per 100 c.c. of media.

The amount of acid given in these tables is always the quantity of total acid contained in 100 c.c. of the 1.0 per cent peptone solution, after addition of one-fifth normal solution of the former. The number of bacteria was calculated from colonies obtained from 1.0 c.c. of culture fluid.

The death-point shows striking differences according to the kinds of acid employed, whether the acidity be determined by the titration method or electrometrically.

If we compare the difference of the death-point in each acid with that of the dissociation-constant and molecular weight of respective acids, no parallelism between these can be traced; they behave entirely independent of each other.

TABLE III

SULPHURIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	4.2	4.46	0		0		0	
2	3.7	4.64	2,940		54,000		5,076,000	4.63
3	3.4	4.72	1,380		14,000		1,512,000	4.72
4	3.2	4.87	3,800		117,000	4.88	25,800,000	5.18
5	2.7	5.37	204,000	5.29	18,600,000	5.40	48,600,000	5.51
6	2.0	5.76	1,728,000	5.70	19,800,000	6.00	78,000,000	6.30
7	1.5	6.50	1,458,000	6.43	25,200,000	6.73	90,000,000	6.88
8	1.0	6.85	7,560,000	6.80	60,000,000	6.93	104,000,000	7.02

TABLE IV

NITRIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	4.2	4.54	0		0		0	
2	3.7	4.63	68		21		0	
3	3.4	4.90	1,080		78,000	4.94	23,400	5.00
4	3.2	5.01	4,560		2,640,000	5.11	16,200,000	5.29
5	2.7	5.23	6,000	5.29	6,600,000	5.30	43,200,000	5.33
6	2.0	5.92	558,000	5.60	27,600,000	5.87	146,000,000	7.20
7	1.5	6.46	4,334,000	6.40	52,800,000	6.43	180,000,000	7.21
8	1.0	6.85	3,600,000	6.70	156,000,000	6.70	171,000,000	7.32

TABLE V

PHOSPHORIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	8.5	4.32	79		0		0	
2	8.0	4.43	660		345	4.48	1,354	4.52
3	7.6	4.48	900	4.50	1,440	4.51	4,600,000	4.69
4	7.2	4.55	1,440		1,500	4.57	18,600,000	4.71
5	6.8	4.76	8,400		25,400	4.76	39,000,000	
6	6.4	4.91	15,300		100,000	4.96	43,800,000	
7	5.4	5.00	78,000		6,600,000	5.00	110,000,000	5.75
8	4.3	5.44	4,200,000		19,200,000	5.58	121,000,000	6.09
9	3.1	6.01	21,000,000	6.07	30,000,000	5.95	164,000,000	6.40
10	1.5	6.63	22,200,000		54,000,000	6.61	150,000,000	7.19

TABLE VI
FORMIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	2.7	5.40	0	5.40	0	5.41	0	5.41
2	2.3	5.63	2,701	5.54	20,000	5.78	4,260,000	5.82
3	2.0	5.80	3,480	5.80	100,000	6.00	33,000,000	7.06
4	1.75	6.02	38,880	6.01	7,200,000	6.28	72,000,000	7.19
5	1.50	6.32	168,200	6.31	43,200,000	6.45	102,000,000	7.22
6	1.25	6.49	858,000	6.50	50,600,000	6.67	138,000,000	7.30
7	1.00	6.80	798,000	6.77	60,000,000	6.92	108,000,000	7.29

TABLE VII
ACETIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	3.7	4.87	11		2		0	
2	3.5	5.00	200		10	5.01	0	
3	3.3	5.18	1,380		44	5.20	1	
4	2.6	5.30	3,900		1,320		208	
5	2.4	5.45	3,910		630,000		25,200,000	5.48
6	2.2	5.85	3,720	5.86	818,000		39,000,000	
7	2.0	6.00	17,600		16,200,000	6.21	102,000,000	6.20
8	1.8	6.18	114,000		42,000,000		99,500,000	6.60
9	1.6	6.34	2,700,000		66,000,000	6.61	108,000,000	6.61
10	1.4	6.65	2,430,000		54,130,000		102,900,000	6.81
11	1.0	6.82	9,000,000		84,000,000	6.84	104,000,000	7.04

TABLE VIII
CHLORACETIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	2.0	5.52	42		0		0	5.54
2	1.8	5.74	55		0		0	
3	1.65	5.90	200		19		0	
4	1.50	6.09	35		60		0	6.10
5	1.35	6.24	1,000		2,380,000	6.18	41,400,000	6.40
6	1.20	6.47	110,000		18,600,000	6.40	156,000,000	6.61

TABLE IX
PROPIONIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	12.0	4.43	8		4	4.43	0	4.44
2	10.0	4.51	10		0	4.50	0	4.53
3	8.0	4.65	7		0	4.67	0	4.71
4	6.0	4.79	10		0	4.75	0	4.82
5	5.0	5.09	5		34		0	5.12
6	4.0	5.35	75		121		6	5.40
7	3.5	5.51	420		23,900		1,700	5.52
8	3.0	5.60	12,700		1,010,000	5.54	12,000,000	5.62
9	2.5	6.10	66,000		15,000,000		60,000,000	6.60
10	2.0	6.60	576,000	6.52	36,700,000	6.67	78,000,000	6.75
11	1.5	6.73	954,000	6.71	72,000,000	6.70	126 000,000	6.98

TABLE X—LACTIC ACID (OPTICALLY INACTIVE)

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	4.5	4.20	19		0		0	4.21
2	4.3	4.45	4	4.46	1	4.46	0	
3	4.0	4.57	1	4.56	0		9	4.59
4	3.8	4.61	1,320		1,223		2,216,500	4.62
5	3.5	4.70	1,380		3,000		2,484,000	4.80
6	3.2	4.91	2,000		500,000		13,800,000	4.94
7	2.7	4.93	4,400		1,200,000		18,000,000	5.60
8	2.0	5.50	5,700		8,000,000		60,000,000	6.01
9	1.7	5.84	380,000	5.80	9,000,000	5.97	62,000,000	6.23
10	1.5	6.18	440,000		15,000,000	6.30	99,000,000	6.34
11	1.5	6.24	1,380,000	6.26	61,000,000	6.26	114,000,000	6.72

TABLE XI—OXALIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	2.2	5.67	0		0	5.67	0	5.66
2	2.0	5.86	0		0	5.87	0	5.87
3	1.8	5.93	1		0	5.98	0	5.99
4	1.6	6.20	0		0	6.30	2	6.30
5	1.4	6.39	600		132,000	6.41	45,000,000	6.46
6	1.2	6.61	8,100		41,400,000	6.70	96,000,000	6.72

TABLE XII—NORMAL SUCCINIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	14.0	4.24	0		0	4.24	0	4.24
2	12.0	4.35	184		140	4.39	17	4.40
3	10.0	4.40	3,240		18,000	4.43	6,000,000	4.49
4	7.0	4.50	213,000		990,000	4.52	13,000,000	4.59
5	5.0	4.70	370,000		5,400,000	4.75	44,400,000	5.38
6	4.0	5.10	2,400,000	5.01	12,000,000	4.80	45,000,000	5.72
7	3.0	5.32	5,000,000		24,600,000	5.39	66,000,000	6.12
8	2.5	5.82	10,200,000		30,000,000	5.94	96,000,000	6.39
9	2.0	6.30	16,270,000		48,000,000	6.45	114,000,000	6.80
10	1.8	6.61	27,000,000	6.54	78,000,000	6.74	114,000,000	6.97
11	1.6	6.73	28,000,000	6.60	84,000,000	6.80	124,000,000	6.99
12	1.5	6.84	26,000,000	6.75	83,000,000	6.80	109,000,000	7.07

TABLE XIII—DEXTROTARTARIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	7.5	4.20	11		0		0	
2	5.0	4.45	100		1,260		210,000	4.48
3	4.25	4.60	300		8,049		21,310,000	4.62
4	3.75	4.80	6,400				33,600,000	
5	3.0	5.00	3,900				37,000,000	
6	2.75	5.20	12,600		18,600,000		55,000,000	5.98
7	2.30	5.35	630,000		47,400,000		90,000,000	5.98
8	2.0	5.71	770,000	5.83	57,000,000		148,000,000	6.33
9	1.5	6.00	19,800,000	6.01	72,200,000		142,000,000	
10	1.0	6.20	30,600,000	6.12	70,000,000		130,000,000	6.75

TABLE XIV
CITRIC ACID

INCUBATION TIME IN HOURS	0		12		24		48	
	ACID (C.C.)	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H	N.B. PER C.C.	P_H
1	9.0	4.40	1,320		99	4.40	0	
2	6.0	4.59	2,200		13,140	4.59	21,000,000	4.61
3	5.0	4.81	4,400		550,000		38,400,000	
4	4.0	4.99	4,200		354,000	5.03	37,000,000	5.12
5	3.0	5.16	11,000	5.10	27,900,000		39,000,000	5.39
6	2.0	6.20	41,000	6.16	28,000,000	6.31	66,000,000	6.72

The results of Experiment II are summarized in Table XV.

TABLE XV(I)

	ACID PER 100 C.C. OF MEDIA	P_H	P_H (AVERAGE)
1. Hydrochloric	4.2- 3.7 (c.c.)	4.51-4.70	4.6
2. Sulphuric	4.2- 3.7	4.46-4.63	4.6
3. Nitric	3.7- 3.4	4.63-4.90	4.8
4. Phosphoric	8.5- 8.0	4.32-4.52	4.5
5. Formic	2.7- 2.3	5.41-4.80	5.6
6. Acetic	3.3- 2.6	5.20-5.48	5.3
7. Monochlor	1.5- 1.35	6.10-6.40	6.3
8. Propionic	4.0- 3.0	5.40-5.62	5.5
9. Norm. Lactic	4.0- 3.8	4.57-4.61	4.6
10. Oxalic	1.6- 1.4	6.20-6.40	6.3
11. Norm. Succinic	12.0-10.10	4.35-4.49	4.4
12. Norm. Tartaric	7.5- 5.0	4.20-4.45	4.4
13. Citric	9.0- 5.0	4.40-4.60	4.5

TABLE XV (II)

ACIDS	DISSOCIATION-CONSTANT (100 k)	MOLECULAR WEIGHT
1. Chloracetic	0.16	94.484
2. Oxalic	4.0	126.048
3. Formic	0.021	46.016
4. Acetic	0.0018	60.032
5. Nitric		63.018
6. Propionic	0.0014	74.048
7. Lactic	0.014	90.048
8. Hydrochloric		36.468
9. Sulphuric		98.086
10. Tartaric	0.097	150.048
11. Citric	0.083	210.08
12. Phosphoric		98.086
13. Succinic	0.0066	118.048

EXPERIMENT III

In the foregoing experiment, I observed that a certain acceleration or delay in development of bacteria may be induced at a low concentration of different acids, so I attempted here to go into detail in this respect; though for this purpose a large number of controls is required, I adopted the following two methods, to obtain a general consideration on this subject.

Method A.—To ninety parts of peptone solution ten parts of buffer mixture (boric acid, KCl and NaOH, after Clark, $P_H = 8.4$)[17] were added to give the solution $P_H = 8.3$. The same series of acids as employed in Experi-

ment II were added in one-fifth normal solution to the above mixture, thus the whole solution attained about $P_H = 7.0$. Here the amount of acids to give final—P_H value differs widely according to their dissociation-constants. Thus we may assume that these solutions consist of exactly the same components, except in their amount of anion (Table XVI).

Method B.—Equal quantities (2.0 c.c.) of one-fifth normal solutions of each acid are added to the peptone water. These mixtures receive further 20.0 c.c. of phosphate solution which is prepared with proper amounts of one-fifth mol solutions of primary and secondary sodium phosphate in various

	ACID	$Na_2HPO_42H_20$	NaH_2PO_4	2% PEPTONE	AQ. DEST.
1. Hydrochloric	2.0 c.c.	12.75 c.c.	7.25 c.c.	50.0 c.c.	28.0 c.c.
2. Sulphuric	"	13.00	7.00	"	"
3. Nitric	"	12.20	7.80	"	"
4. Phosphoric	"	15.64	4.36	"	"
5. Formic	"	12.00	8.00	"	"
6. Acetic	"	12.05	7.95	"	"
7. Propionic	"	13.50	6.50	"	"
8. Lactic	"	11.70	8.30	"	"
9. Oxalic	"	11.80	8.20	"	"
10. Succinic	"	13.00	7.00	"	"
11. Tartaric	"	12.10	7.90	"	"
12. Citric	"	11.90	8.10	"	"

20.00 c.c. in all.

TABLE XVI

INCUBATION TIME IN HOURS	18	24	36	48	AFTER TEN DAYS
ADJUSTED BY	NUMBER OF BACTERIA PER C C.				P_H
1. Hydrochloric	60,000,000	127,000,000	155,800,000	128,400,000	7.99
2. Sulphuric	48,780,000	70,000,000	96,600,000	114,000,000	8.08
3. Nitric	98,400,000	145,400,000	164,200,000	142,000,000	8.12
4. Phosphoric	51,300,000	63,000,000	96,000,000	113,000,000	7.59
5. Formic	39,000,000	48,180,000	72,000,000	72,600,000	8.37
6. Acetic	55,800,000	58,800,000	85,200,000	109,800,000	8.10
7. Propionic	37,200,000	63,000,000	91,000,000	92,400,000	7.97
8. N. Lactic	34,000,000	51,600,000	66,000,000	83,900,000	8.14
9. Oxalic	47,000,000	90,000,000	91,600,000	73,100,000	7.87
10. N. Succinic	44,400,000	62,000,000	87,000,000	104,400,000	8.19
11. N. Tartaric	63,600,000	78,000,000	114,000,000	63,000,000	8.20
12. Citric	46,800,000	69,600,000	84,000,000	64,800,000	7.88

TABLE XVII

INCUBATION TIME IN HOURS	18	24	48	72
ADJUSTED BY				
1. Hydrochloric	33,600,000	108,000,000	165,000,000	198,000,000
2. Sulphuric	25,000,000	78,000,000	79,000,000	96,000,000
3. Nitric	58,000,000	138,000,000	169,600,000	202,000,000
4. Phosphoric	49,000,000	60,000,000	72,000,000	88,000,000
5. Formic	19,200,000	54,000,000	76,800,000	118,000,000
6. Acetic	50,400,000	68,400,000	114,000,000	156,000,000
7. Propionic	21,000,000	76,800,000	96,000,000	99,000,000
8. N. Lactic	18,600,000	54,100,000	83,500,000	117,000,000
9. Oxalic	40,400,000	94,200,000	96,000,000	133,000,000
10. N. Succinic	25,000,000	87,600,000	98,000,000	103,000,000
11. N. Tartaric	28 200,000	54,000,000	106,000,000	149,000,000
12. Citric	27,600,000	66,000,000	68,000,000	126,000,000

proportions, to bring the reaction of the whole mixture to $P_H = 7.0$ (Table XVII).

Tables XVI and XVII lead us to the following conclusions. In these tables the bacteria are given in average numbers from repeated experiments, and Table XVI shows a remarkable difference in number of bacteria according to acids added to the culture fluid. The development of bacteria is accelerated by some inorganic acids, e.g., nitric and hydrochloric acid; among them the former has the most favorable effect upon bacterial development. On the contrary the growth of bacteria is remarkably retarded by addition of sulphuric and phosphoric acid. On the other hand no striking difference in action may be noticed among organic acids, the accelerating power also being much less than that of nitric or hydrochloric acid. Moreover the end-reaction of the fluid is in close relationship with the kinds of acid employed. The result of Table XVII coincides on the whole with that of Table XVI. The end-reaction suffers no remarkable change from the buffer action of a large quantity of phosphoric acid. Detailed descriptions are omitted here.

From these results it is obvious that nitric acid gives particularly good results as a growth-promoting agent, when it is added in proper amount to the media, at least this is true with colon bacilli, and hence nitric acid should be employed in adjusting the reaction of media for B. coli.

EXPERIMENT IV

It was already stated by Rosenblatt and Rozenband[18] in 1907 that acetic acid stands in the way of alcoholic fermentation. Dam[19] recognized the same fact with lactic acid. On the other hand, Clark, Wyeth and Scheer[20, 21, 22] gave some accounts of the effect of these acids upon the final H-ion concentration by the alcoholic fermentation of B. coli.

These latter authors are of the opinion, that, when such acids as may be produced in the course of fermentation already exist from the beginning of cultivation, the fermentation decreases considerably in consequence of the retarding action of acids added.

It seems incorrect to estimate the process of fermentation by way of determination of the end hydrogen-ion concentrations, as for instance, colon bacilli which may perish during the fermentation by the acids which they produced themselves. However, from facts mentioned above we must bear in mind, that besides H, another important factor in the acid-action should not be neglected. This is anion. It has led me to attempt again to examine the end-P_H of culture media, which have previously been adjusted to the P_H of 7.0 with the series of acids used in my foregoing experiments.

The media used for this purpose were prepared like those employed in Exp. III. B., to which glucose was added to give a 2.0 per cent solution.

The final P_H given in Table XVIII is that of five to seven days after the inoculation when no live cells in the media could be detected. The results thus obtained from my experiment show approximate agreement with those of Clark and others as to hydrochloric, acetic and lactic acid. However, not

TABLE XVIII

	FINAL P_H (5-7 DAYS)
1. Hydrochloric	4.56
2. Sulphuric	4.56
3. Nitric	4.63
4. Phosphoric	4.58
5. Formic	4.62
6. Acetic	4.63
7. Propionic	4.68
8. N. Lactic	4.62
9. Oxalic	4.56
10. N. Succinic	4.63
11. N. Tartaric	4.63
12. Citric	4.62

only these three kinds of acids but also another series of acids, as given in Table XVIII, may show the same.

The differences in the end-reaction of media differ somewhat widely in the reports of Clark and others. This is probably due to the difference in quantities of acid and buffer solution.

EXPERIMENT V

This experiment was made in order to know the results of neutralizing of media with equivalent quantities of NaOH to the bactericidal concentration of acids.

TABLE XIX

	P_H (INITIAL)	N.B. (1 DAY)	N.B. (2 DAYS)	P_H (10 DAYS)
1. Hydrochloric	7.40	101,000,000	90,000,000	7.91
2. Nitric	7.40	144,000,000	122,000,000	7.95
3. Tartaric	7.40	86,000,000	78,000,000	8.20
4. Acetic	7.23	96,000,000	90,000,000	8.00
5. Oxalic	7.41	64,000,000	90,000,000	8.01
6. Chloracetic	7.38	89,000,000	48,000,000	7.81

Peptone solutions are reduced to coagulation, which is more or less reversible by the addition of acid or alkali. About this fact Kligler[23] reports as follows: "It is a well-known fact that when media are neutralized to phenolphthalein, a precipitate is produced which may be partly redissolved by the addition of acid. If this precipitate is filtered off, the nutritive value of the medium is appreciably lowered." Among the kinds of acids employed in my experiments, oxalic acid was the one which caused the irreversible coagulation of peptone solution. After addition of the acid I added the same amounts of alkali (NaOH), but the precipitate was never redissolved. Notwithstanding this property, the nutritive value was never affected if no filtering operation was made such as Kligler performed.

EXPERIMENT VI

This experiment was attempted to gain some aspects about the results obtained with mixtures of equal parts of two different acids, which are quite different from each other in their bactericidal concentrations.

TABLE XX

(OXALIC + TARTARIC		P_H	N.B. (24 HRS.)
1	3.5 (c.c.)	4.70	0
2	3.0	5.10	0
3	2.9	5.20	1
4	2.7	5.23	11
5	2.5	5.48	1,008,000
6	2.0	6.0	18,080,000
7	1.5	6.51	22,680,000
8	1.0	6.95	34,800,000

	$\frac{N}{5}$ ACID (C.C.)	P_H (DEATH-POINT)
(Oxal. Tart.)	2.9-2.5	5.2
Oxalic	1.6-1.4	6.3
Tartaric	7.5-5.0	4.4

Oxalic and tartaric acid were used for this purpose.

BY	DEATH-POINT
Oxalic acid	$P_H = 6.3$
Tartaric acid	$P_H = 4.4$
Acid mixture	$P_H = 5.3$

These results are brought out more clearly in Table XX. It is particularly interesting to note the approach of the death-point of both acids towards each other. This may be, in other words, a case of buffer-action of weak acid.

EXPERIMENT VII

Much study has been bestowed by many investigators on the effects of so-called neutral salts upon bacterial growth. Lately Sherman and Holm[24] published some notes thereon and said: "A strain of B. coli which showed no growth at P_H of 4.8 (adjusted by HCl) in 1.0 per cent peptone solution at 37° C., grew quite readily in the same medium to which NaCl had been added to make a 0.2 mol solution. On the other hand, Na-citrate shows no widening effect upon the P_H zone of growth and even narrows the limits of H-ion concentration at which this strain will grow."

At first, I doubted these widening effects of NaCl, which have been emphasized by these authors, thinking that the results may probably be due to the so-called "common-ion-effect," through which the ionization of the acid, here HCl, was affected and correspondingly caused the lowering of the H-ion concentrations. Secondly, I attempted to solve, just by means of this fact, whether the acids play a rôle on the bacteria as anion or as undissociated molecules.

As shown in Table XXII acetic acid clearly displays its bactericidal action as undissociated molecules.

As shown in Table XXI, the addition of NaCL to 0.1 mol causes apparently not only the shifting of limits of growth which were attained by the use of HCl, but also a decided acceleration in growth of bacteria, 0.3 mol NaCl being rather less effective. In this point Sherman is right. But there is no agreement of results obtained by Sherman and myself, when Na-citrate has been added to obtain 0.2 mol solution. In my case bacterial growth was observed after addition of Na-citrate, though it has no such accelerating effect as NaCl. It may, of course, be taken into consideration

TABLE XXI

CONTROL

	$\frac{N}{5}$ HCL	P_H	N.B. 48 HR.
1	4.8 (c.c.)	4.25	0
2	4.5	4.34	0
3	4.3	4.50	0
4	3.6	4.69	60,000

I

	$\frac{N}{5}$ HCL	NaCL	P_H	N.B. 24 HR.	48 HR.
1	4.8 (c.c.)	01. M	4.32	10,231,000	37,800,000
2	4.5	"	4.40	17,400,000	43,000,000
3	4.3	"	4.60	30,600,000	44,400,000
4	3.6	"	4.75	34,000,000	184,000,000

II

	$\frac{N}{5}$ HCL	NaCL	P_H	N.B. 24 HR.	48 HR.
1	4.8 (c.c.)	0.3 M.	4.33	268	300
2	4.5	"	4.42	227	480,000
3	4.3	"	4.64	203,000	3,060,000
4	3.6	"	4.78	34,800,000	162,000,000

III

	$\frac{N}{5}$ HCL	Na-CIT.	P_H	N.B. 24 HR.	48 HR.
1	4.8 (c.c.)	0.2 M.	6.94	33,000,000	30,000,000
2	4.5	"	7.02	36,000,000	30,000,000
3	4.3	"	7.10	51,000,000	–
4	3.6	"	7.25	58,000,000	–

TABLE XXII

	$\frac{N}{5}$ ACETIC	Na-ACET.	P_H	N.B. (48 HR.)
1	3.4 (c.c.)	–	5.03	0
2	"	0.01 Mol	5.53	0
3	"	0.05	5.81	40
4	"	0.1	6.12	0
5	"	0.2	6.36	600
6	"	0.3	6.49	6,340

TABLE XXIII

CONTROL

	$\frac{N}{5}$ CITRIC A.	P_H	COLONIES (24 HR.)
1	9.0 (c.c.)	4.41	3
2	8.0	4.48	2,520
3	6.0	4.60	130,000
4	5.0	5.01	4,860,000

	$\frac{N}{5}$ CITRIC A.	Na-CIT.	P_H	N.B. (24 HR.)
1	9.0 (c.c.)	0.1 M.	6.22	24,400,000
2	8.0	"	6.30	65,400,000
3	6.0	"	6.41	55,000,000
4	5.0	"	6.53	88,200,000

With citric acid its P_{H+} may be of rather more importance, as shown in Table XXIII.

TABLE XXIV

CONTROL

	Na-FORMATE	P_H	N.B. (24 HR.)
1	0.01 (M.)	7.50	30,000,000
2	0.05	7.59	33,000,000
3	0.1	7.68	27,000,000
4	0.2	7.71	25,200,000
5	0.3	7.75	16,200,000
6	0.4	7.81	8,580,000
7	0.5	7.89	960,000

	$\frac{N}{5}$ FORMIC A.	Na-FORMATE	P_H	N.B. (24 HR.)
1	2.7 (c.c.)	–	5.42	11
2	"	0.01 (M.)	5.51	0
3	"	0.05	5.59	0
4	"	0.1	5.72	4
5	"	0.2	5.90	14
6	"	0.3	6.11	127
7	"	0.4	6.16	67
8	"	0.5	6.21	46

It is clear from this Table XXIV, that formic acid also displays its toxic action upon bacteria as undissociated molecules.

TABLE XXV

CONTROL

	Na-OXALATE	P_H	N.B. (24 HR.)
1	0.001 (M.)	7.31	163,800,000
2	0.005	7.52	101,400,000
3	0.01	7.62	182
4	0.05	7.99	44,400,000
5	0.1	8.37	6,800,000
6	0.2	8.89	0

	$\frac{N}{5}$ OXALIC A.	Na-OXALATE	P_H	N.B. (24 HR.)
1	2.0 (c.c.)	–	6.24	0
2	"	0.01 M.	6.75	4
3	"	0.05	7.31	42,300,000
4	"	0.1	7.68	16,200,000

that a simultaneous lowering of H-ion concentration of media, owing to the slight alkalinity of Na-citrate solutions, may occur.

As may be deducted from the results in Table XXV, Na-oxalate acts upon bacterial growth in quite a different way, as compared with other sodium salts of various acids. While smaller amounts of these salts display strong toxicity, larger amounts show rather less toxic power, but further increase of the salts inhibits the growth of bacteria again. Some notes about these facts may shortly be given as follows:

1. When these salts are used in very small amounts, the amount of oxalic-ion too will be small, consequently they can display almost no toxic effects.

2. A moderate amount of this salt will reduce a moderate quantity of oxalic-ion, so it may act much more toxic accordingly.

3. Further increase in the amount of salts may cause the decrease of toxicity because of the strong increase in alkalinity, the dissociation of salt-

molecules might be depressed by the presence of anion of some other electrolytes.

4. The total inhibition of bacterial growth by the larger amounts of these salts is apparently due to the strong alkalinity.

SUMMARY AND CONCLUSIONS

From the foregoing experiments we may summarize the results as follows:

1. The nature of acidification of media at a certain phase should not always be ascribed to the existence of any sugars in the media. Most probably it comes from the decomposition of protein substances by bacteria.

2. The final H-ion concentrations show remarkable differences according to the kinds of acids.

That is to say, they differ in death-point (Table XV, Table XVIII) and in degree of alkalization when acids were added in small amounts. Furthermore, the bacterial growth never runs parallel with this degree of alkalization of media (Tables XVI, XIX). Accordingly, we wish rather to emphasize the difference in value of acids as a buffer. Formic acid is most liable to alkalization, phosphoric and oxalic acids least. With lactic acid the result of my experiment differs from that obtained by Koser.

3. Many acids vary in their accelerating or retarding effect on bacterial growth, when added in low concentrations. With inorganic acids, the presence of nitric as well as hydrochloric acid stimulates the growth of bacteria, while sulphuric and phosphoric acids effect delay in growth. On the other hand, organic acids have, as a whole, no stimulating effect, except acetic and oxalic acid which, however, act far inferiorly to nitric or hydrochloric acid. So it appears quite correct, at least with colon bacilli, that culture media should be adjusted with nitric acid, should any acidifying be necessary.

4. With media which contain sugars there exists a certain relationship between the final reaction and the kind of acids employed for adjustment of initial reaction. Now we may understand much about the conditions which induce a different final P_H when different sugars are used.

5. The nutrient value of peptone is scarcely altered by the coagulating action of acid or alkali.

6. The effect of acid upon microorganisms should not only be attributed to its H-ion concentrations but also to its anion and further to its undissociated molecules, as stated already by Krönig and Paul.

Furthermore, it should be remembered that the acid-action upon microorganisms is quite specific; even one and the same acid may act differently on different microorganisms.

To sum up, in biologic researches, in which any culture of microorganisms is made and the optimum H-ion concentration or death-point of bacteria is discussed, we must always, besides CH^+, give some account in detail as to the kinds of acid or alkali and their concentration used (in other words normality).

I wish to express here my sincere thanks to our Director, Prof. Dr. Nagayo

and Doctors Tamiya, Imamura and Kojima, who were kind enough to give me helpful assistance and advice in the course of this work.

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KOLMER'S CHOLESTERINIZED VERSUS ACETONE INSOLUBLE ANTIGEN*

By Wilfred H. Kellogg, M.D., Berkeley, California

A PREVIOUS study of the Kolmer modification of the Wassermann test, carried out particularly with a view of clearing up certain discrepancies between laboratories in their reports on the same specimens, which discrepancies seemed at times to be related to differences in the antigen, has been published.†

At the present time I want to report on a further series of comparative tests totaling nearly 10,000. In the former article results of a series of 506 consecutive blood specimens tested by the Kolmer method using Kolmer's antigen, and by the Kolmer method using acetone insoluble antigen, and also tested by another method using the same two antigens, were reported. This made four tests in all for each serum. Some of the results are briefly summarized as follows:

Nine serums were markedly stronger in the Kolmer test with acetone insoluble antigen than they were in the same test with Kolmer's antigen, and the same serums gave a stronger reaction in the other test with the acetone insoluble antigen than they did with the Kolmer showing that the difference was in the antigen and in favor of the acetone insoluble type.

*From the State Hygienic Laboratory, University of California, Berkeley, California.
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†Kellogg, et al: A Study of the Kolmer Method of Complement Fixation for the Diagnosis of Syphilis, Jour. Lab. and Clin. Med., April, 1924.

Seventeen serums were just the opposite; that is, they gave stronger reactions in both types of Wassermann test with the Kolmer antigen than they did in the same test with the acetone insoluble antigen, again demonstrating the difference to be the antigen but this time in favor of Kolmer's cholesterinized antigen.

Of the above tests two were 4-plus in Kolmer's test and negative in the same test using the other antigen, while three were 4-plus in the Kolmer test using acetone insoluble antigen and only one plus when Kolmer's antigen was used. One was 3-plus with the acetone insoluble and negative with the Kolmer antigen, in both instances the Kolmer method being used. Table I shows that the advantage is with the cholesterinized antigen, excepting that there are a few more 4-plus reactions with the acetone insoluble than with the cholesterinized antigen.

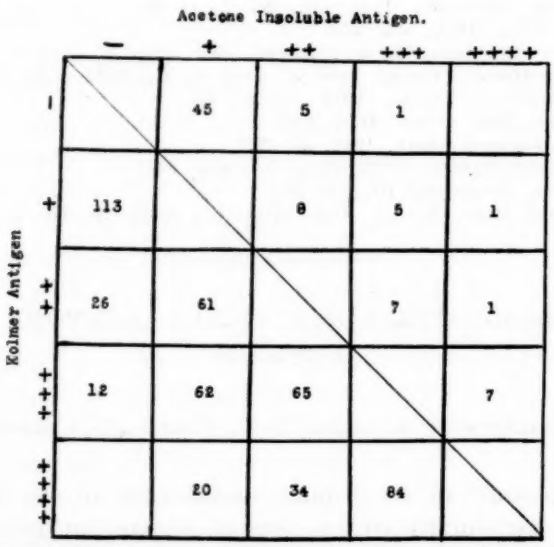

Fig. 1.—Each individual serum of the number shown in any square gave the result shown at the top of the column for the acetone insoluble antigen and at the left with the Kolmer antigen. Consequently, all serums placed above the diagonal line were stronger with the acetone insoluble antigen and those below the line were stronger with the Kolmer antigen.

TABLE I

DEGREE OF REACTION	KOLMER TEST		OTHER TEST	
	WITH KOLMER ANTIGEN	WITH ACETONE INSOLUBLE ANTIGEN	WITH KOLMER ANTIGEN	WITH ACETONE INSOLUBLE ANTIGEN
–	10	32	11	20
+	19	13	24	14
++	15	8	12	5
+++	20	9	25	13
++++	26	28	15	36
Anticomplementary	0	0	3	2

Based on the above observations the conclusion was reached that variation in antigen is the greatest single cause for disagreement between laboratories, and that there are some syphilitic serums giving a positive reaction with cholesterinized antigen and a negative or a weaker reaction with the acetone insoluble type and vice versa.

At this time, as a result of the testing of a further series of 9307 specimens, the conclusion is reached that the difference in reaction with the two types of antigen as above noted is not so important, as indicated by the results obtained in the smaller series of tests. In the present series of 9307 specimens, all were tested twice by the Kolmer method, one test with Kolmer's antigen and the other with acetone insoluble antigen. There were 2023 serums giving a positive reaction of greater or lesser degree in one or both tests. Conflicting results, principally as to the degree of reaction were obtained with 557 of this number. The diagram (Fig. 1) is arranged to show these differences graphically.

It will be noted that among the serums that were negative with Kolmer's cholesterinized antigen, there were 45 that were 1-plus, 5 that were 2-plus, one that was 3-plus and none that were 4-plus with the acetone insoluble antigen. Of those that were negative with the acetone insoluble antigen 113 were 1-plus, 26 were 2-plus, 12 were 3-plus, and none were 4-plus with the Kolmer antigen. It is concluded, therefore, as a result of this more extended series of observations that, while the difference in reaction of different serums to the two types of antigen is definitely demonstrated, it is not the important factor that seemed likely from the first series of tests. The use of two antigens and the double testing of serums is not demonstrated as a necessity but one source of conflicting reports from different laboratories is clearly shown and should be borne in mind by clinicians. The greater sensitivity as compared to the acetone insoluble type, of the cholesterinized antigen, in the form used by Kolmer, is shown.

THE PREPARATION AND CLINICAL TESTS OF AN ANTISTREPTOCOCCUS (SCARLATINAE) SERUM*

By N. S. Ferry, Ph.B., M.D., R. W. Pryer, M.S., Dr.P.H., and L. W. Fisher, B.S., Detroit, Michigan

IN January, 1924, Dochez[1] described an antitoxic serum, for the treatment of scarlet fever, which was tested out clinically and reported on by Blake, Trask and Lynch.[2] This serum was obtained from a horse immunized to the Streptococcus scarlatinae by a special method said by Zingher[3] to be carried out by injecting into the cellular tissues of the neck of the horse a small amount of liquefied agar and into this nodule of agar, after it has solidified, the sedimented live organisms. The bacteria, thus protected from the injurious phagocytic action of the leucocytes of the blood of the animal, are supposed to produce a toxin which, in turn, stimulates the formation of antibodies. According to Zingher, "One of the objectionable features of this method of animal inoculation is that a large sloughing ulcer is produced at the site of the injected mass of agar, which is discharged as a foreign body." Blake and his associates described the

*From the Medical Research Laboratories, Parke, Davis & Co., and the Detroit Department of Health Laboratories.

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results of their clinical tests of this serum, as follows: "In twelve cases of scarlet fever, five of which were severely toxic, administration of the serum has been followed by rapid and complete recovery within from twelve to thirty-six hours. In all but the two most severe cases, a single intramuscular injection of from 40 to 60 c.c. of serum has sufficed. In the two extremely toxic cases approximately 200 c.c. was required. Whether the serum will prove to be of value in late cases with septic complications remains to be determined. In the single case of this kind in which the serum has been tried, a single injection of 40 c.c. had no beneficial effect on the complications, even though it brought about a temporary disappearance of the rash."

In April, 1924, the Dicks[4] described the preparation of a scarlet fever antitoxin, of considerably more promise, carried out by injecting a horse with toxin obtained from the filtered water of condensation of blood agar growths of the Streptococcus scarlatinae. It was found that 10 c.c. of this serum contained antitoxin sufficient to neutralize twenty times the amount of toxin that previously had produced nausea, vomiting, general malaise, fever and a definite scarlatinal rash in a susceptible adult. Clinical results with this serum have not been reported.

Zingher described a toxin, prepared in a manner differing from that of the Dicks, produced by growing the organisms in a 5 per cent horse blood broth and filtering, after being incubated about five days. It is understood that an antitoxin serum is being prepared from this toxin.

It is obvious that these antiserums prepared by Dochez, the Dicks and others, by the immunization of the horse with the toxin from the Streptococcus scarlatinae, are purely antitoxic in nature and action. While they will neutralize the toxin produced by the organisms, it is doubtful whether they have any effect upon their invasive properties.

If scarlet fever is caused by the Streptococcus scarlatinae, the symptoms no doubt, are the result of both the invasion of the organisms in question and the exotoxin produced by them; the toxin causing the rash and other symptoms indicative of toxemia, while the invasion of the organisms results in adenitis, arthritis, otitis media and other complications often accompanying the rash but usually found in the later stages of the disease. While an antitoxic serum should neutralize the toxin and, if given in the very earliest stages, prevent the rash and bring about a rapid recovery in many cases, it is a question whether it would effect in any way the invasive properties of the organism. An antibacterial serum would be of decidedly more advantage in the latter case but such a serum alone would not be expected to prove of much value in the extremely toxic cases.

The purpose of this paper is to report, in a more or less preliminary way, the preparation and clinical results from the use of a Streptococcus scarlatinae antiserum prepared in such a way as to endow it with both antibacterial and antitoxic properties. To produce this serum, horses were injected with the live organisms as well as the toxin. It was thought that a serum of this type would not only neutralize the toxin but would be more likely to retard or check the invasion of the organisms than a straight antitoxin serum. While it is recog-

nized by the authors that there are no known satisfactory laboratory methods of determining the relative value of such a serum; nevertheless, the following tests were carried out, which furnished the data upon which the deductions are based, to the effect that the serum has both antibacterial and antitoxic properties. Agglutination and complement-fixation tests were carried out, similar to those required of other antibacterial sera, with positive results. The serum was used repeatedly in a large number of cases to neutralize the toxin for the control mixture of the Dick test with perfectly satisfactory results, showing that it was capable of neutralizing the toxin of the Streptococcus scarlatinae. The serum also gave the rash fading test of Schultz-Charlton[5] which corroborates the previous test, proving its antitoxic properties.

The most striking results, however, and after all the most important, were those following the clinical tests. Of the thirty cases treated to date at the Herman Kiefer Hospital, Detroit, with the assistance of H. C. Crissman, Chief Resident Physician, twenty-two cases had a fall in temperature to normal within twenty-four to thirty-six hours with a marked improvement in all symptoms. Five cases resulted in a fall of temperature within two to three days with a slower general improvement, while three cases gave no evidence of being influenced in any way by the serum. No deaths were reported in this series.

As a control, an equal number of cases were treated at the same time with other experimental sera as well as with convalescent human serum and normal horse serum. Practically the same results were obtained with the serum under discussion as with the convalescent human serum. All of these sera except the convalescent serum were treated by number only, thus entirely eliminating the personal equation from the results.

As a result of a severe epidemic of smallpox in the city, all unvaccinated hospital cases were vaccinated upon admission so that many of the cases under treatment, especially some of those not giving the best results, were suffering at the same time with active "takes." This, no doubt, had a tendency to maintain the temperature above normal longer than otherwise would have been the case. It is felt, also, that had the serum been given in larger doses and injected intramuscularly, similar to the method practiced by Blake, even better results would have been obtained. The dose ranged from 15 c.c. to 30 c.c. given subcutaneously, while the dose of the Dochez serum given by Blake ranged from 40 c.c. to 60 c.c. and even to 200 c.c., given intramuscularly.

The following is a résumé of some of the case reports:

CASE 11659.—Age twelve, female; admitted April 25, 1924. Onset April 24. Remarks: Had third degree burns on hands April 19. Very sick. Heavy rash. Delirious. April 26, received 30 c.c. serum. Results: Temperature 103.4° dropping to normal next day and remaining there.

CASE 11669.—Age twenty-eight, female; admitted April 26, 1924. Onset April 23. Remarks: Very toxic. Marked rash. April 26, received 45 c.c. serum. Results: Temperature 104.4° on 1st day; 101.6° on 2nd and 3rd days. Normal on 4th day and remained.

CASE 11931.—Age twenty-two, male; admitted June 23, 1924. Onset June 20. Remarks: Fairly sick. Heavy rash. June 23, received 30 c.c. serum. Results: Temperature normal next day.

CASE 11932.—Age nine, female; admitted June 23, 1924. Onset June 21. Remarks: Heavy rash. Quite sick. June 23 received 30 c.c. serum. Results: Temperature normal on second day.

CASE 11934.—Age seven, male; admitted June 23, 1924. Onset June 21. Remarks: Quite sick. Faint rash. Temp. 104°. June 23 received 30 c.c. serum. Results: Temperature from 104° to 99.2° next day.

CASE 11752.—Age seven, male; admitted May 11, 1924. Onset May 9. Remarks: Acutely ill. Heavy rash. May 12, received 15 c.c. serum. Results: Temperature May 11, 103.5°; May 13, 99.8°; May 14, normal.

CASE 11758.—Age twenty-six, female; admitted May 14, 1924. Onset May 12. Remarks: Acutely ill. Heavy rash. May 15, received 30 c.c. serum. Results: Temperature 102.4° on May 14th to normal May 16th.

CASE 11696.—Age thirty, male; admitted May 2, 1924. Onset April 29. Remarks: Rash covers entire body. Throat red and swollen. May 3, received 25 c.c. serum. Results: Temperature dropped from 102° to 99° in 24 hr.

CASE 11959.—Age twenty-three, female, admitted July 6, 1924. Onset July 4. Remarks: Heavy rash. Temp. 104° July 7, received 30 c.c. serum. Results: Temperature July 7, 104°; July 8, 102.2°; July 9, 100.2°; July 10, normal.

In order to increase the potency of this serum and at the same time eliminate as far as possible urticarial rashes which usually follow in a certain proportion of cases injected with horse serum, it has been concentrated by the precipitation of the globulins according to a method somewhat similar to the preparation of other well-known concentrated serums. Agglutination tests show that this concentration has increased the titer of the serum about six times. While this does not necessarily imply that its protective value has increased to that extent, it is, more or less, an index of the concentration of the antibodies present. Taking into consideration the results usually following the concentration of antidiphtheritic and antitetanic serum, this increase in antibody content is also about what should be expected. So far the few cases upon which this concentrated serum has been tested have given extremely favorable results with 10 c.c. doses. These cases will be reported later.

CONCLUSIONS

1. An antistreptococcus scarlatinae serum has been prepared with both antitoxic and antibacterial properties.

2. This serum has proved of value as a therapeutic agent in scarlet fever.

3. The serum has been concentrated to at least six times its original strength with favorable clinical results.

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THE EFFECT OF CONSTIPATION AND CATHARSIS ON UROBILINURIA*

By R. S. Dean, M.D., Cleveland, Ohio

EXAMINATION of the urine for urobilin has never been useful in the clinic, partly because of the obscure nature of this neglected pigment, and also because of the enormous variation in amount shown by individual cases. The influence of constipation and catharsis upon the variation in individual cases is the theme of this study. It is hoped that this and similar studies may throw some light on the physiology and pathology of urobilin and urobilinogen—for, unless otherwise stated, these two substances are here studied as one—and thus remotely come to bear on the problems of liver function and hemoglobin. This particular study certainly bears directly on the question of absorption of bile and stercobilin from the intestine, which has recently been reopened by Whipple,[1] who with good evidence asserts that no such absorption occurs. Through an understanding of the effect which constipation and catharsis have on the excretion of urobilin, much may perhaps be learned of the distribution and production of this obscure pigment.

That constipation and catharsis influence the output of urobilin in the urine was observed by several of the earlier writers on this subject. Special mention should be made of the work of Gerhardt[2] and Hildebrant.[3] Most of the early work was done without quantitative observations, but many of the recent investigators have ignored the influence of such simple factors on urobilinuria.[4, 5, 6]

METHOD

The methods used for the present work are those outlined by Wilbur and Addis,[4] with the following modifications:

Instead of grinding the feces in a mortar, the twenty-four hour specimen was placed in an ordinary three quart glass butter churn, which was attached to a 1/40 H.P. electric motor. Water was added to make a volume of 2000 c.c. This quantity of water was used because it was found that a fine, even emulsion could thus be more easily made, and the number 2000 made calculations easy. The hand spectroscope used by Wilbur and Addis was discarded in favor of the usual three-tube laboratory spectroscope (Bunsen and Kirchoff model), which showed the absorption bands at a higher dilution and consequently gave a more exact end-point. The Wilbur and Addis calculations are based on the number of dilutions required to obliterate the urobilin spectroscopic band, plus the number of dilutions required to obliterate the urobilinogen spectroscopic band. The urobilin band practically always disappears last when feces are examined; and the urobilinogen band always disappears last when urine from patients with pathologic urobilinuria is examined. In the present study calculations have been made from the number of dilutions required to obliterate both spectroscopic bands. This modification was used by Blankenhorne[1] while studying the

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distribution of the biliary elements in jaundice. He concluded that the comparative results of the two methods did not vary enough to be of any importance. When these modifications are used, the results presented are numerically different from those obtained by using the identical methods of Wilbur and Addis.

With these modifications the following estimate of the output of urobilin in the feces of two normal persons of regular gastrointestinal habits gave the following results:

Case I		Case II	
	UROBILIN IN FECES UNITS		UROBILIN IN FECES UNITS
Oct. 4	35,000	Nov. 30	25,000
" 5	52,000	Dec. 1	25,000
" 6	60,000	" 2	32,000
" 7	66,000	" 3	25,000
" 8	38,000	" 4	Lost
" 9	38,000	" 5	25,000
" 10	52,000	" 6	38,000
" 11	44,000	" 7	25,000
		" 8	25,000

The uniformity of the daily output of urobilin in the stools of the two cases presented above is attributable in part to the mechanical method of comminution and extraction. Much wider variations were found when the feces were ground in a mortar.

In febrile patients with urobilinuria, advantage was taken of the fact that constipation is usually present. In some cases paregoric was given over a number of days to insure it. Magnesium sulphate was the cathartic used. Calomel, castor oil, and the vegetable cathartics were also tried but they proved to have no special advantage.

In presenting the data from the following cases graphs have been made of the number of units of urobilin in the urine on successive days. In all cases the amount of urobilin excreted in the feces was determined. The results are not shown on the graphs because of the confusion necessarily arising from so much charting.

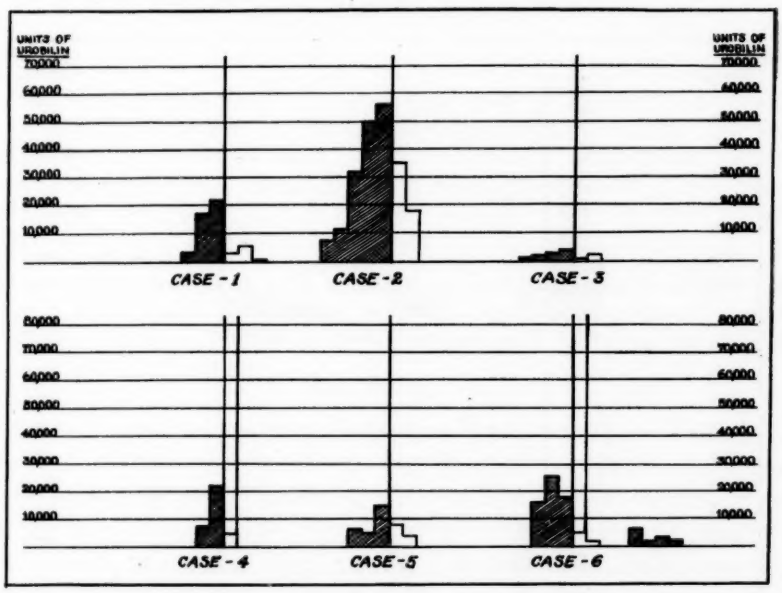
Case I.—Lobar pneumonia. The patient was admitted with consolidation of the right upper and middle lobes. Two days later signs of consolidation were present over the left lower lobe. On the fourth day after admission there was a definite crisis. On the same day there was a definite urobilinuria, which had not been present before. Graph 1 shows the output for six successive days after the onset of urobilinuria. During this time the temperature showed a daily variation of 37°-38°. During the first three days of constipation, the curve shows very rapid rise in urobilinuria. There was one defecation without catharsis on the following day, which was accompanied by a great decrease of urobilinuria.

Case II.—Lobar pneumonia. The patient was admitted on the second day of his illness. The entire right lung was consolidated. There was a crisis on the sixth day. For six days following the crisis the temperature fluctuated between 37° and 38°. Graph 2 shows the urobilinuria from the fourth to the twelfth day of the disease. Three days of constipation are accompanied by a pronounced increase of urobilinuria. The curve shows that this increase persists after the crisis and is greatly reduced by catharsis. The reduction in urobilinuria for the first day of catharsis is much greater than is shown in the

graph, because the cathartic was not given until after the greater part of the urine for that twenty-four-hour period had already been excreted.

CASE III.—Bronchopneumonia. The patient was admitted on the fifth day of the disease. There was an irregular fever reaching 40°. He suffered from severe pleuritic pains in the right side, and chest signs of consolidation were present over small areas below the right clavicle and at the right base posteriorly. The sputum was bloody but not rusty. No evidence of a pneumococcus infection could be found by examination of the sputum or by blood culture. Graph 3 shows the excretion of urobilin from the sixth to the eleventh day of the disease. The output of urobilin in the urine was very low throughout. The first four days of constipation are accompanied by a small but definite increase in urobilinuria. With the resumption of defecation on the fifth day, urobilinuria is reduced. A great contrast is evident between Graph 3 and Graphs 1 and 2. In Graph 3 the urobilinuria is very small on the first day of constipation and is increased only a little by

Graph 1. Graph 2. Graph 3.



Graph 4. Graph 5. Graph 6.

Chart 1.

Cross-hatched areas represent days of constipation.
Clear areas represent days of evacuation.
Perpendicular lines separate periods of constipation and evacuation.
The two perpendicular lines in Graph 4 and Graph 6 represent catharsis on two consecutive days.

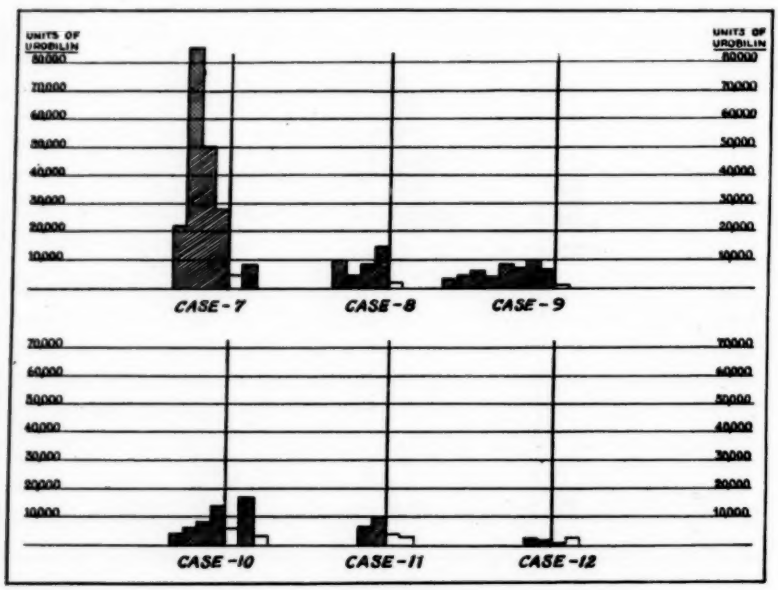
four days of constipation. Defecation at this point reduces urobilinuria slightly. In Graphs 1 and 2 the urobilinuria is much greater at the beginning of constipation than in Graph 3, and there is a very great increase accompanying subsequent constipation. Graphs 1 and 2, in contrast with Graph 3, also show a greater reduction when catharsis took place.

CASE IV.—Lobar pneumonia. The patient was admitted on the fifth day of the disease. There was consolidation of the right upper lobe. The patient had taken a cathartic with results on the day of admission. Graph 4 shows the output for the following six days. After two days of constipation a cathartic was given. Active catharsis is accompanied by a great reduction of urobilinuria on the first day, and continued catharsis on the following days is accompanied by absence of urobilin in the urine.

CASE V.—Advanced Hodgkin's disease. Pronounced involvement of all the external lymph nodes was present. The afternoon temperature was usually near 39.5°. At a later date postmortem examination showed also extensive involvement of the liver. Graph 5 shows an increase of urobilinuria on the third day of constipation. Daily bowel movements without catharsis on the two following days are accompanied by a decrease in urobilinuria, with no definite change in the temperature curve.

CASE VI.—Lobar pneumonia. The patient was admitted after he had been ill one week. The entire left lung was consolidated. Graph 6 shows urobilinuria, which is increased by constipation, abolished by catharsis, and reappears with subsequent constipation. The patient was febrile on the first, second and third days. The temperature was normal on the fifth, sixth and seventh days. On the eighth day the temperature was 38°, and on the ninth day, 36.5°. Thereafter the temperature was normal. Besides the effect of catharsis and constipation, the temperature appears to affect urobilinuria. This is shown most distinctly on the eighth, ninth and tenth days.

Graph 7. Graph 8. Graph 9.



Graph 10. Graph 11. Graph 12.

Chart 2.

CASE VII.—Lobar pneumonia. The patient was admitted on the second day of his illness. The right lower lobe was consolidated. There was no bowel movement on the second day of his illness, and this study was begun on the third day. On this, the third day of constipation, Graph 7 shows a very large increase of urobilinuria, followed by a decrease on the fifth and sixth days. Catharsis given on the seventh day is accompanied by a further decrease. On the eighth day there was no bowel movement and the urobilinuria again increases. The temperature changes may exp'ain the decrease without catharsis on the fifth and sixth days, as there was a definite fall of temperature on the fifth day and a further fall accompanying the crisis on the sixth day.

CASE VIII.—Pseudoleucemia. The clinical diagnosis was confirmed by autopsy. The chief clinical findings were profound anemia, general adenopathy, a large spleen, and slight enlargement of the liver. There was an irregular fever. Graph 8 shows some irregularity of output regardless of catharsis and constipation. It should be mentioned that on the

first day of constipation the fever was higher than on any other day represented in the graph. Catharsis on the fifth day is accompanied by a marked decrease in urobilinuria.

CASE IX.—Tuberculous peritonitis. There was an irregular fever. With daily bowel movements there was a low grade urobilinuria. Constipation of eight days' duration was induced by giving paregoric. Urobilinuria was definitely increased by this prolonged constipation, but the increase was small and irregular as compared with that in cases showing a greater output of urobilin in the urine with daily defecation. Catharsis on the ninth day almost freed the urine of urobilin. The study was repeated in the same patient with similar results.

CASE X.—Pulmonary tuberculosis. The onset of the disease was acute. The temperature was continuously near 39°. Graph 10 shows an increase of urobilinuria accompanying four days of constipation. On the fifth day there is a decrease accompanying defecation without catharsis. On the sixth day with no bowel movement urobilinuria again increases to its highest level represented in the graph. On the seventh day it again falls accompanied by defecation. It is possible that the large amount of urobilin in the urine on the sixth day was due in part to absorption from contents in the intestinal tract which had collected on the days of constipation, and were only partly evacuated on the fifth.

CASE XI.—Pernicious anemia. The anemia was severe with a regular afternoon rise of temperature to 39°. The study of urobilinuria was not begun until after the patient had been constipated for three days. Graph 11 shows a moderate degree of urobilinuria reached after five days of constipation, and on the sixth day it is reduced to a very low level by moderate catharsis.

CASE XII.—Anemia of unknown origin. There were regular daily defecations with slight urobilinuria. Graph 12 shows a slight but definite reduction of urobilinuria caused by catharsis. On the day following the catharsis, there was one bowel movement, and the urobilinuria resumes, approximately, the same level as on the days preceding catharsis.

DISCUSSION

Besides the cases presented, others were studied. They similarly showed an increase in urobilinuria during constipation and a decrease with catharsis. Among them was a case of cirrhosis of the liver, but in this instance on repeated trials the increase and decrease of urobilinuria were not always present. This lack of constancy may have been due to irregularity of absorption from the intestine. That intestinal absorption was interfered with in this patient was shown by the repeated recurrence of ascites after tapping. This case is mentioned as the only one in which there was found any departure from the rule that constipation increases urobilinuria and catharsis decreases it.

In lobar pneumonia it must be remembered that there is a period of maximum urobilinuria in the course of the disease.[9] The increase and decrease might be confused with changes associated with constipation and catharsis. With this possibility in view, the effect of constipation and catharsis upon urobilinuria was studied in all stages of lobar pneumonia. The results in the different stages differed only in degree, the urobilinuria being modified most by constipation and catharsis when output was greatest.

From several of the cases presented, it has appeared that the presence or absence of fever had a decided effect on urobilinuria. This question was investigated separately and will be reported upon subsequently.

The specific gravity of the urine was taken in every case to determine whether the decrease in urobilinuria following catharsis was due to a lack of

excretion by the kidneys. If catharsis were drastic enough to decrease the amount of urine excreted, the specific gravity should be increased. In all cases it was found that any definite decrease in the amount of urine excreted following catharsis was accompanied by an increase of specific gravity and a reduction of urobilinuria.

In all cases red blood counts, hemoglobin estimations and hematocrite readings were made to determine the possibility of blood destruction as the origin of urobilinuria. These data are not discussed here because the present study concerns itself only with the excretion of urobilin.

These observations show that constipation exaggerates pathologic urobilinuria. The resumption of daily bowel movements reduces this exaggerated urobilinuria to its former level. Catharsis even of moderate degree causes a very pronounced decrease of urobilinuria. The amount of increase attending constipation depends to a large extent on the amount present before constipation began. A high-grade urobilinuria with daily bowel movements is increased much more by constipation than a low-grade urobilinuria under similar conditions. The converse is true of catharsis, for while low-grade urobilinuria is decreased only slightly by catharsis, a high-grade urobilinuria is greatly reduced. Although, owing to other factors, it is difficult to make an exact statement of how much the excretion of urobilin in a given case is affected by constipation and catharsis, a rough estimate may be given. If a patient excretes 15,000 units of urobilin in the urine daily with daily bowel movements, three days of constipation may be expected to increase the daily excretion to 30,000 or 45,000 units. Catharsis at this point sufficient to produce three good bowel movements could be expected to reduce the daily output to between 3,000 and 10,000 units.

These facts suggest that one factor which determines the degree of pathologic urobilinuria is the length of time that the intestinal contents remain in the intestinal tract. When large quantities of urobilin are excreted in the urine, a considerable portion is probably collected from the contents of the colon. It is a striking fact that the degree to which urobilinuria is affected by constipation and catharsis is largely determined by the degree of urobilinuria with daily bowel movements prior to the constipation or catharsis.

CONCLUSIONS

Constipation exaggerates a pathologic urobilinuria. Evacuation of the bowel diminishes it.

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LABORATORY METHODS

STUDIES ON LOCAL ANESTHESIA. I

THE USE OF TÜRCK'S REFLEX METHOD TO DETERMINE THE EFFICIENCY FACTOR OF LOCAL ANESTHETICS*

By W. J. R. Heinekamp, M.S., M.D., Chicago, Ill.

SINCE the skin of the frog is somewhat similar to mucous membrane, it has been used to test the efficiency of local anesthetics. The method, as used, is based on Türck's method of recording reflex time and consists in placing the frog's leg about midway to the knee in a bath of the anesthetic and determining the influence of the drug on the reflex time at definite intervals by stimulating the foot with dilute hydrochloric acid. The frog is decapitated to simplify the reflex. In this series, however, the heart was neither removed as by Sollman[1] nor tied as by Zorn.[2] The fact that the lymph hearts still beat after these procedures presumes that the absorption of the drug is going on and makes this operation unnecessary. Clinically, there is absorption when local anesthetics are applied to mucous membrane, and since this method is an attempt to simulate clinical conditions, removal of the circulation is of questionable value and not indicated. If some absorption occurs, it has no practical influence on the method, since the local action is predominant.

The stimulus used was hydrochloric acid. As shown in Chart 1, the reflex time is dependent on the strength of the stimulus until a point is reached when, regardless of the concentration of the acid, the reaction time remains constant. Any strength above N/5 HCl causes a reflex in one second, that evidently being the minimum reflex time. The strength of the acid is of great importance since a strong solution, e. g., N/1 HCl, such as was used by Issekutz,[3] is corrosive and destroys the sensory nerve endings, or at least alters their activity. An acid in such a concentration is in itself an anestheticum dolorosum. This probably accounts for the results Issekutz obtained.

On the other hand, a minimal stimulus is objectionable in that the reaction time becomes more variable the weaker the acid used (see Chart 2). Hence, to be most reliable the acid should be strong enough to give constant results and weak enough to be noncorrosive. A weaker acid has a decreased osmotic pressure and, hence, would allow a greater flow of the drug from the tissue into the bath. Tenth-normal HCl seems to be best suited for this purpose since the responses are somewhat constant and the acid is not corrosive in this strength for the time it is used.

The length of time the anesthetic acts is of importance since frequent stimulations require considerable washing, which permits and aids the recuperation

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of the partially anesthetized skin. A one per cent cocaine hydrochloride solution will paralyze the sensory nerve endings as determined by this method, if allowed to act ten consecutive minutes. However, if the reflex is tested at the end of five minutes and again at ten minutes the paralysis is not complete although the reflex is slowed. We have, therefore, taken ten minutes as the time between tests, realizing that in some cases the result may vary almost this length of time as will be discussed later.

EXPERIMENTS

Designating ten minutes as the time necessary for one per cent cocaine hydrochloride to totally inhibit a reflex with N/10 HCl used as a stimulus (see summary). I found that the drugs used in one per cent solutions have the following comparative values, as shown in the subjoined protocols and tables.

The following protocols exemplify the experiments.

PROTOCOL I

COCAINE 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	10 min.
	Left Leg	10 min.
2		
	Right Leg	10 min.
	Left Leg	10 min.
3		
	Right Leg	10 min.
	Left Leg	10 min.
	Average	10 min.

PROTOCOL II

HOLOCAINE 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	10 min.
	Left Leg	10 min.
2		
	Right Leg	10 min.
	Left Leg	10 min.
3		
	Right Leg	10 min.
	Left Leg	10 min.
	Average	10 min.

PROTOCOL III

ALYPIN 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	10 min.
	Left Leg	10 min.
2		
	Right Leg	10 min.
	Left Leg	10 min.
3		
	Right Leg	10 min.
	Left Leg	10 min.
	Average	10 min.

PROTOCOL IV

BUTYN 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	10 min.
	Left Leg	10 min.
2		
	Right Leg	10 min.
	Left Leg	10 min.
3		
	Right Leg	10 min.
	Left Leg	10 min.
	Average	10 min.

PROTOCOL V

SALIGENIN 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	10 min.
	Left Leg	10 min.
2		
	Right Leg	10 min.
	Left Leg	10 min.
3		
	Right Leg	10 min.
	Left Leg	10 min.
	Average	10 min.

PROTOCOL VI

STOVAINE 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	20 min.
	Left Leg	20 min.
2		
	Right Leg	20 min.
	Left Leg	20 min.
3		
	Right Leg	20 min.
	Left Leg	20 min.
	Average	20 min.

PROTOCOL VII

TROPACOCAINE 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	20 min.
	Left Leg	20 min.
2		
	Right Leg	20 min.
	Left Leg	20 min.
3		
	Right Leg	20 min.
	Left Leg	20 min.
	Average	20 min.

Protocol VIII

BETA-EUCAINE 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	20 min.
	Left Leg	20 min.
2		
	Right Leg	20 min.
	Left Leg	20 min.
3		
	Right Leg	20 min.
	Left Leg	20 min.
	Average	20 min.

Protocol IX

APOTHESINE 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	60 min.
	Left Leg	60 min.
2		
	Right Leg	60 min.
	Left Leg	60 min.
3		
	Right Leg	60 min.
	Left Leg	none
	Average	60 min.

Protocol X

NOVOCAINE 1 PER CENT

FROG		TIME OF ONSET OF ANESTHESIA
1		
	Right Leg	No Anesthesia in one hour
	Left Leg	No Anesthesia in one hour
2		
	Right Leg	No Anesthesia in one hour
	Left Leg	No Anesthesia in one hour
3		
	Right Leg	No Anesthesia in one hour
	Left Leg	No Anesthesia in one hour

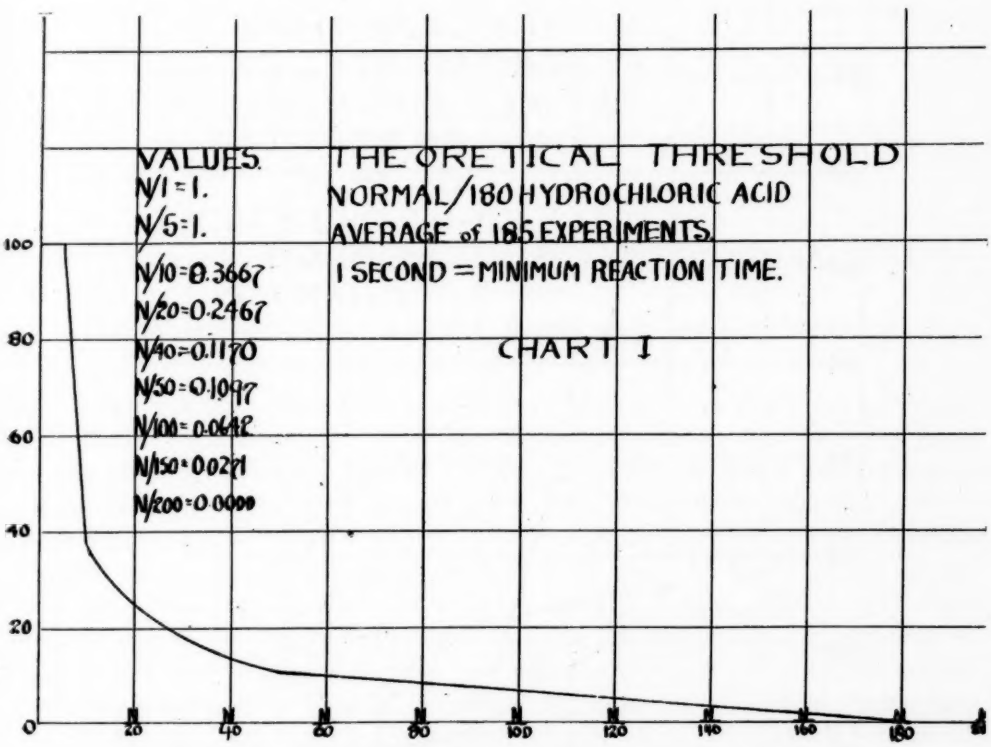
Summary

DRUG	EFFICIENCY FACTOR
Cocaine hydrochloride	1
Holocaine	1
Alypin	1
Butyn	1
Saligenin	1
Stovaine	0.5
Tropacocaine	0.5
Beta-Eucaine	0.5
Apothesine	0.1666
Novocaine	0.0000

DISCUSSION

While this method of determining the relative anesthetic power of various solutions is used, the results are so variant that not much reliability can be placed upon them.

It must be borne in mind that this method measures only the activity of local anesthetics on mucous membrane. Novocaine, which is probably the safest and one of the most efficient local anesthetics in injection work, is practically without action when applied to mucous membrane. (Hence, the only conclusion that can be drawn from the results obtained by the use of this method is that the drug under consideration is or is not a good anesthetic for mucous membrane. Other factors such as toxicity, corrosiveness, etc., are disregarded.)

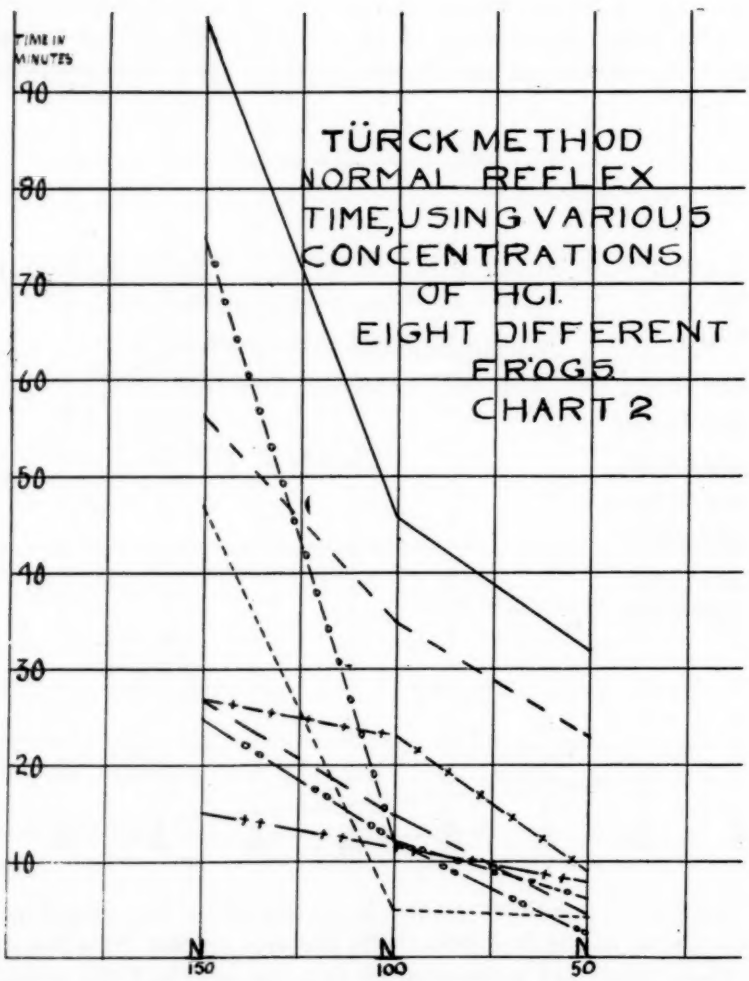


The duration of anesthesia cannot be measured by this procedure. While the nervous system of the frog apparently recovers quickly from the shock produced by decapitation, we must consider that the cord and the medulla suffer some molecular disintegration which predisposes to their inactivity. This is noticed even more when the circulation is tied off, since the nervous elements then react quickly to asphyxia and starvation. It cannot be concluded that the frog is still under the influence of the anesthetic when a reflex is not obtained. The nervous system might be dead. Hence, this method does not furnish any data concerning the duration of the anesthetic.

In a certain number of frogs, regardless of the amount of brain destroyed, swimming movements that simulate the normal reflex are present. It is pos-

sible for the skin to be completely anesthetized and still have swimming movements result on immersion of the foot into the acid. Hence, a false reflex may alter the efficiency factor as determined by this method.

The time is not accurate, in that nine minutes difference may actually be present in the anesthetizing time of two drugs while the efficiency factor would be the same. For example, drug A does not paralyze in ten minutes but does in twenty. There is no way of determining whether paralysis was effected in eleven or nineteen minutes.



We found that ten minute intervals were optimum for the determination of the reflex time. Cocaine, which we used as a standard, did not paralyze in ten minutes if the reflex was taken at five minutes and again at ten minutes. It is necessary for cocaine to act ten consecutive minutes. Apparently the washing removes enough of the drug so that the action is deferred. Hence, a ten minute interval is necessary. It is not improbable that a drug failing to act in ten minutes but producing paralysis in twenty minutes, would effect complete anesthesia if allowed to act fifteen consecutive minutes.

Since frogs react differently in various seasons we feel that the results depend in part on the time of year the experiments were completed.

Chart 2 shows the results obtained from eight experiments done under the same conditions. The wide variations in reactions increase as the strength of the acid becomes weaker. Hence, we feel that any conclusions must be based on the average of a large number of experiments. Chart 1 is a curve based on a series of 185 experiments. We find that while N/180 HCl is not the absolute threshold, very few frogs react with it as a stimulus.

CONCLUSIONS

While this method is simple and easily performed the disparity in results shows that reliable data cannot be obtained. The method is of little value in the comparative work and of questionable value from a practical standpoint.

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A METHOD FOR SAMPLING AND ANALYSIS OF STOMACH GAS*

By A. D. Dunn, M.D., and F. L. Dunn, M.D., Omaha, Nebraska

In a study of the carbon dioxide and oxygen content of stomach gas in normal persons,[1] we endeavored to learn the volume per cent ratios of these two gases in gas aspirated from the stomach of healthy individuals under varying conditions. It was shown that atmospheric air introduced into the stomach tends to approach equilibrium with the blood gases within one hour in the case of carbon dioxide, and considerably later in the case of oxygen. In this work we repeatedly met with unaccountable sudden variations in the volume per cent of carbon dioxide, and oxygen in successive samples from the fasting stomach obtained at intervals of five to twenty minutes. The explanation for these sudden variations would seem to lie either in (1) swallowed air, or (2) in sudden regurgitating of duodenal gas, or (3) in indeterminable factors affecting the rates of liberation of carbon dioxide, from and the absorption of oxygen into the stomach wall, or (4) in possible partitioning of the gas bubble, or (5) in two or more of these factors combined. From roentgenologic investigations, appreciable duodenal regurgitation of gas would seem to be negligible. In any event, whatever gas might be passed from the duodenum into the stomach should in normal individuals at least approximate the composition of stomach gas. The one factor susceptible of experimental control is the swallowing of air; a study of this factor involves a brief review of methods heretofore used in the investigation of stomach gas.

Planer (1860)[2] was the first to make any accurate attempt at the analysis

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of stomach gases. His work was confined to the examination of gas samples obtained from the stomachs and intestines of dogs recently killed.

Schierbeck[3] repeated Planer's experiments and studied the gases found in the stomach and intestines of human bodies immediately after death. He tried to estimate the carbon dioxide in the living by bubbling the gas from stomach contents aspirated with water through a standard barium hydroxide solution. His last method has to do only with gas which can be driven off from the stomach contents and does not apply to gas contained in the stomach apart from food.

Hoppe-Seyler[4] collected gas by connecting a three-stoppered inverted bottle filled with water with a stomach-tube. The gas was collected over water and transferred to a Hempel burette for analysis. Later he collected gas directly into a tube, transferring it to a Hempel burette and analyzing it by Hempel's method. This work was done almost entirely on pathologic material and his normal gas values indicate that air swallowing was not controlled.

Woodyatt and Graham[5] first introduced room air into the normal stomach, withdrawing it in five minutes for analysis. This method was used merely to establish the liberation of carbon dioxide into the stomach by the gastric mucosa.

Yllpö[6] studied the changes taking place in air, carbon dioxide and oxygen introduced into the fasting stomach. A 1 cm. stomach tube was used both to introduce and to sample gas. The difficulty of *voluntarily* controlling air swallowing was mentioned but no *mechanical* means was employed to overcome this error. His average normal value, 4.4 per cent carbon dioxide, indicates that air swallowing played a not unimportant rôle.

Kantor[7] used the rather crude Hoppe-Seyler method in a "few preliminary estimations undertaken on an unselected series of individuals aspirated at various intervals after eating" in which no mention was made of the unknown factor of air swallowing. His low carbon dioxide values suggest that equilibrium of this gas with air swallowed with the sampling tube, had not been reached before samples were removed for analysis.

It was obvious from a cursory review of the literature that reliable quantitative methods for the study of stomach gas obtained from living human subjects had not yet been developed. No attempt had been made to determine the rate of gaseous interchange within the stomach. Little effort had been made to learn the effect of swallowed air on stomach gas concentrations. In upwards of three hundred gas analyses made on fifty different individuals, normal and abnormal, we have developed a method which seems to meet the requirements of sound experimental procedure for obtaining and analyzing stomach gas. The important features of this method are the introduction of a measured volume of air of known composition into the stomach, the restriction of sampling to the fasting stomach in studying normal gaseous interchange, the prevention of air swallowing, and sampling at frequent intervals.

The introduction of a measured quantity of air of known composition into the stomach in order to facilitate sampling has obvious advantages. Difficulty in sampling from insufficient air bubbles is common. The average size of the air bubble is about 50 to 100 c.c. Its position is often out of range of the sam-

pling bucket. One hundred fifty to two hundred c.c. of air introduced into the stomach insures several adequate samples. The taking of a deep breath by the descent of the diaphragm will often make a gas bubble accessible to the sampling bucket. The introduction of air into the stomach is also of value in determining

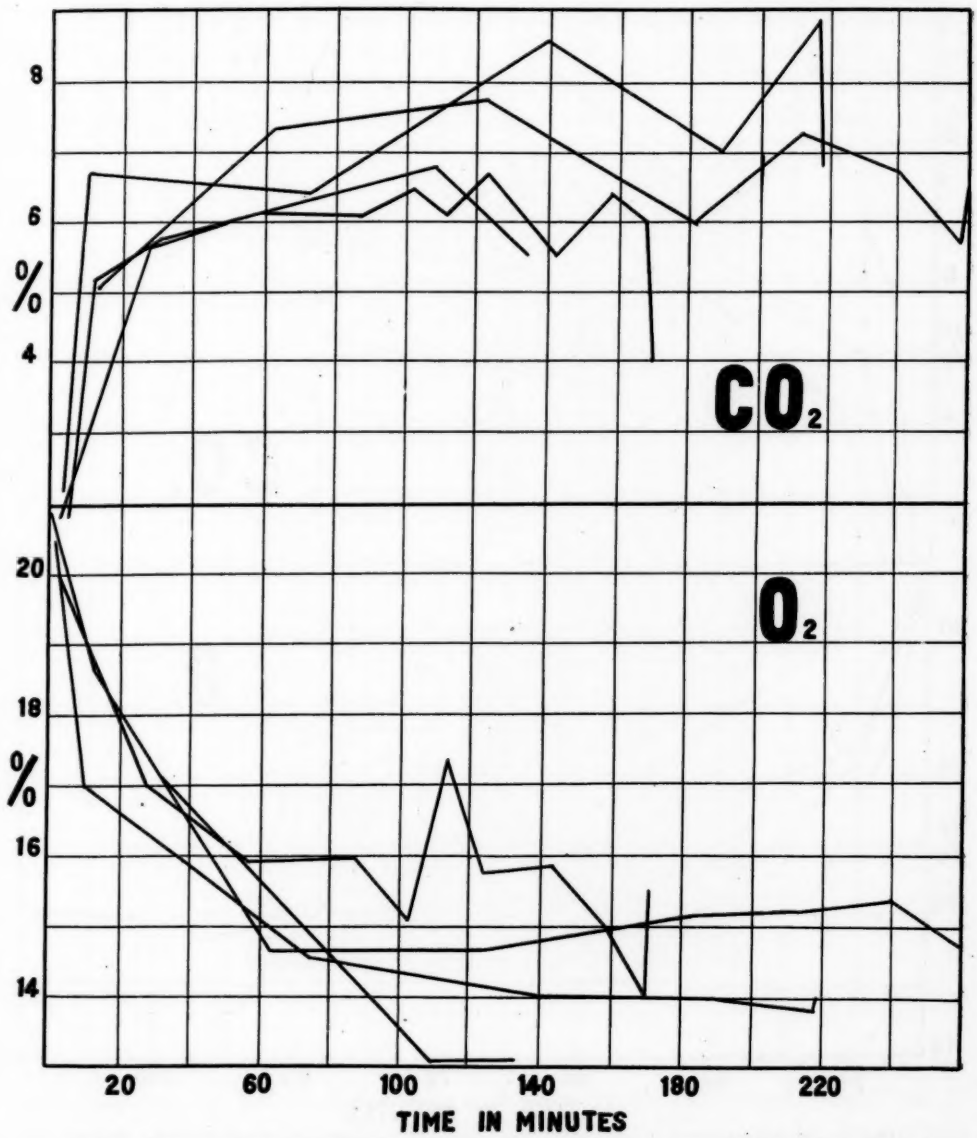

Fig. 1.—Four curves showing sudden variations frequently encountered when only voluntary efforts are made to control the swallowing of air. It is to be noted that the greater variations occur in the carbon dioxid curves.

the rate of liberation of carbon dioxide into, and of absorption of oxygen from, the stomach.

Sampling is done largely on the fasting stomach. The admission of food soils the apparatus, tends to disturb timed sampling and introduces unnecessary variables such as the possible effects of postprandial variations in alveolar air

as described by Dodd.[8] Inasmuch as it would seem at first desirable to determine the rate of liberation of CO_2 into the stomach, the advantages for this purpose of the fasting stomach are clear. However, a series of analyses made from digesting stomachs of the same subjects, failed to reveal noteworthy differ-

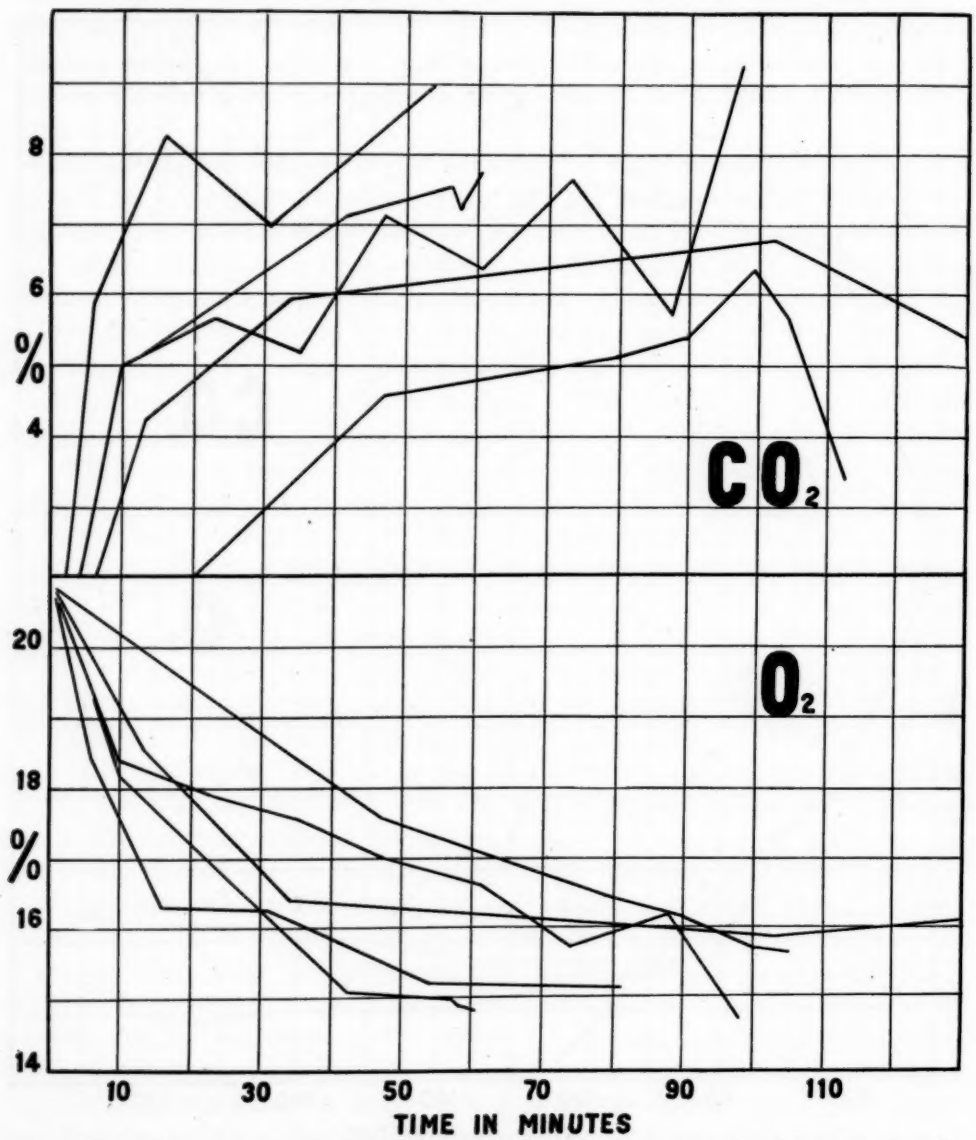

Fig. 2.—The lack of smoothness in the curves is similar to that noted in Fig. 1. An effort was made to prevent ingress of air into the stomach by constant pumping of saliva and air out of the esophagus.

ences in the percentages of carbon dioxide and oxygen. Fermentation is a minor if not a negligible factor in the production of stomach gas, except possibly in the absence of free hydrochloric acid. In the study of pathologic conditions, it will undoubtedly be necessary to determine with accuracy what effect the presence of food in the stomach may have on gas production. For the sake of sim-

plicity, however, it seemed wise for the present to confine our work to the fasting stomach.

Swallowing of air must be controlled. Only in exceptional instances have we been able to obtain curves of expected smoothness without mechanical prevention of air swallowing. Fig. 1 shows the irregular curves usually obtained when a single Rehfuss tube was used in sampling and only voluntary efforts made to control air swallowing. There is an unconscious passage of air and saliva into the esophagus which cannot be satisfactorily controlled by voluntary effort. We first attempted to get rid of swallowed air by cementing a per-

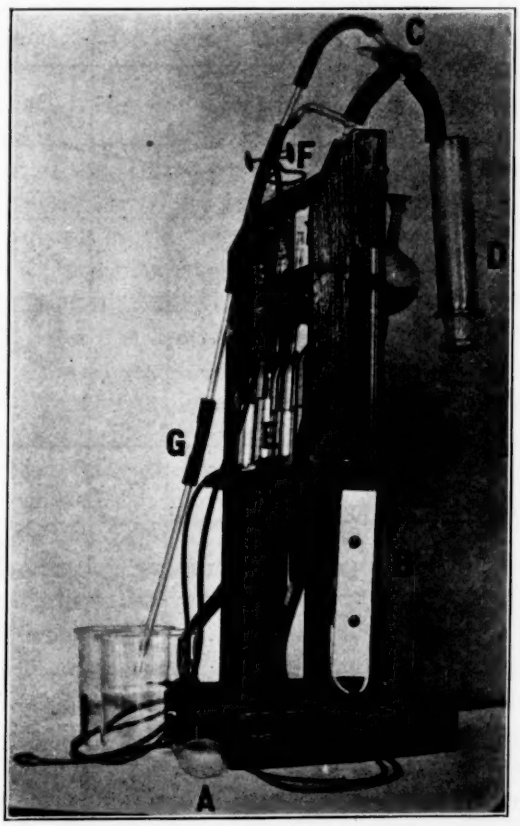

Fig. 3.—Sampling apparatus with balloon to prevent air swallowing, description to be found in the text.

forated duodenal tube to the aspirating tube. This second tube was fixed to the aspiraing tube so that its lower end, which was freely perforated, would rest in the lower esophageal ampulla. It was attached to a water suction pump and negative pressures of 40 to 100 mm. of mercury were used. Fig. 2 shows that the results obtained by this method were no better than by the single tube method. It still seemed that air might enter the stomach in spite of suction as air incorporated in small bubbles in saliva. A distensible rubber balloon placed just above the cardia seemed the most likely solution of the swallowed air problem. X-ray studies indicated that the balloons should have a distensibility of 3 to 5 cm. A balloon pressure of 25 to 50 mm. of mercury depending on the

elasticity of the balloon was needed. It is likely that the balloon acts somewhat after the manner of a water lock, at times permitting accumulated saliva to pass, carrying minute bubbles of air, to be sure, but apparently not in sufficient

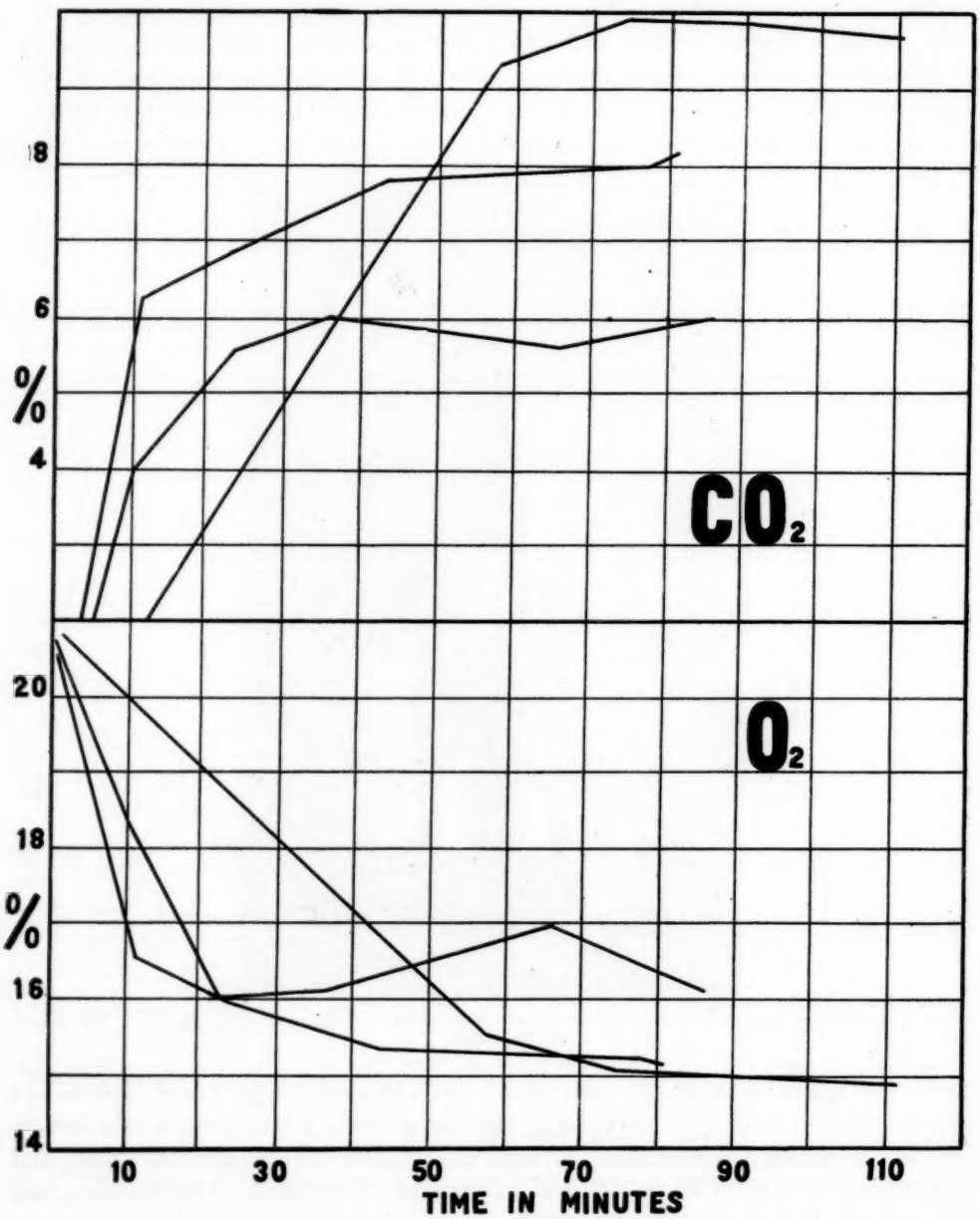

Fig. 4.—Typical illustrations of the curves obtained when air swallowing is prevented by the distensible esophageal balloon.

amount to vitiate results. The discomfort to the subject is little if any more than that experienced in fractional test meals or nonsurgical biliary drainage. Fig. 3 shows apparatus with aspirating bucket and balloon with sampling tube

and manometer attached. Fig. 4 shows characteristic curves obtained by this method.

In brief the method of obtaining gas is as follows: Fig. 3 illustrates the collecting apparatus ready for use. It consists of a Rehfuss tube with a small balloon (*A*) attached about 10 cm. from the bucket, a manometer and test tube (*B*) connected to the balloon, a three-way stopcock (*C*), *Luer* syringe, (*D*) and sampling tubes (*E*) for collecting gas samples. The balloon is tested for leaks and for the pressure required for the desired distension. The tube is introduced to 42 to 48 cm. at which level most gas bubbles can be tapped. The balloon is then inflated. The stopcock (*C*) is turned so that Rehfuss tube and syringe are in communication, the clamp (*F*) removed and gentle suction is applied. Fasting contents are then drawn into the syringe for fermentation tests, the stopcock (*C*) is turned so that syringe and waste pipe (*G*) are in communication and the fasting contents forced into a test tube. The syringe and Rehfuss tube are then put in communication and fasting contents removed until the gas bubble is tapped. Then the stopcock (*C*) is turned so syringe (*D*) and waste pipe (*G*) are shut off, the stopcock on the sampling tube opened, and the gas sample collected by lowering the mercury reservoir. If insufficient gas is obtained or if so desired 150-200 c.c. of gas of known composition is introduced via *G C D C A*. After the collection of each sample, acidulated water (½ per cent sulphuric acid) is run into the Rehfuss tube and its connections until all the air has been displaced. As shown in Fig. 3 each of the sampling tubes (*E*) also contain 10-15 c.c. acidulated water. By this means the samples are collected uncontaminated. In transferring the collecting apparatus from one sampling tube to the next the clamp (*F*) is closed. Samples are collected at five to thirty minute intervals as desired.

A Pettersen-Palmquist apparatus was first used for gas analysis, but on account of the large amount of gas necessary (25 c.c. for each analysis) and on account of the desirability of analyzing successive samples obtained at brief intervals of time this machine was discarded in favor of a Boothby modification of the Haldane Gas Analysis machine for carbon dioxide and oxygen. Although the latter machine is accurate to a few hundredths of one per cent, no attempt has been made to get results better than correct to one-tenth of one per cent. When a sufficient sized sample was collected the mixing method described by Boothby and Saniford[9] was used. When the sample was too small Haldane's[10] original technic of displacing all the air in the system by mercury was employed.

COMMENT

It is apparent that the methods heretofore used in the study of stomach gases are open to criticism. The study of gas as obtained postmortem or from freshly killed or anesthetized animals cannot be expected to give the same representative values as when obtained from subjects sitting quietly in a chair or lying in bed. Furthermore, no effort has been made to determine whether the samples collected from living human subjects were representative of the equilibria for these subjects or whether such samples consisted appreciably of air swallowed in the sampling process. Given an accurate method of obtaining and

analyzing stomach gas, much work must be done before a conception of the physiologic pathology of the symptom of "gas on the stomach" can be formulated. The method opens up many problems for investigation. From our limited observations on supposedly normal subjects with the above method, it would seem that in atmospheric air introduced into the fasting stomach, the carbon dioxide rises rapidly and that in twenty to thirty minutes the curves flatten out and hold somewhere between 6 and 9 volumes per cent. In other words there is a stomach respiration. Many observations must be made in order to establish the limits of the normal curves if such misconceptions as once prevailed in regard to hydrochloric acid are to be forestalled. The behavior of stomach gases in disease is relatively unknown. While we have made numerous studies of patients complaining of stomach gas and obtained results of interest, yet sufficient observations have not been made to venture any conclusions. The reaction of the stomach to the introduction of various gases such as carbon dioxide, oxygen, nitrogen, etc., should also be of interest.

CONCLUSIONS

1. A more accurate method has been described for the collection and analysis of stomach gas with reference to its carbon dioxide and oxygen content, permitting of a study of the equilibrium relationships existing in normal subjects.

2. Analyses made to date show that in air of known composition introduced into the stomach of supposedly normal subjects the carbon dioxide and oxygen contents tend to come into equilibrium at relatively constant values.

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A MODIFICATION OF THE MOIST CHAMBER*

By Alfred Reich, Cleveland, Ohio

A SIMPLE and economical device for the study of tissue, fungi, and micro-organisms in general, *in vitro,* over a prolonged period of time, and under high magnification, can be made by grinding holes five-eighths of an inch in diameter, through the bottom of a Petri dish, and sealing large cover slips over the openings at the outer surface by means of balsam or some other adhesive material (Fig. 1). The dish is then covered and placed, inverted, in a large Petri dish, which is in turn covered with adhesive tape and placed in an autoclave. On removal from the sterilizer, the device, which throughout these operations is kept in a horizontal position with the inner Petri dish inverted, should be placed in a cool atmosphere to permit the balsam to congeal.

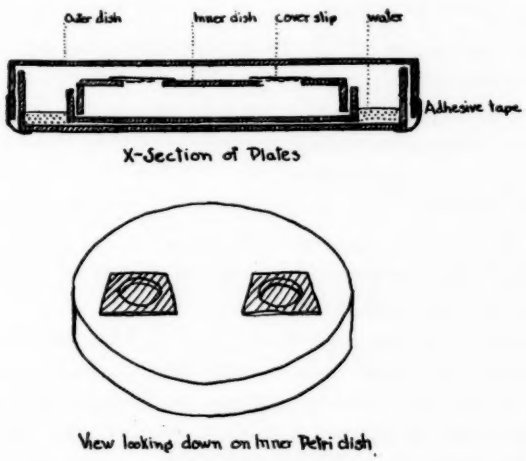

Fig. 1.

To use this device, the inner dish is removed from the larger one, and placed upright on a flat surface. By lifting the cover of the dish as one does to streak a plate, a thin film of the desired liquid medium may be spread into one of the ground-out recesses of the dish by means of a sterile platinum loop or capillary pipette. The medium may then be inoculated with the desired organisms, after which the smaller Petri dish is replaced in its original inverted position in the larger one. A small quantity of water is then placed in the larger dish to prevent the evaporation of the medium, the cover is again secured with adhesive tape, and the whole incubated at a suitable temperature. The effect is the same as that secured by the well-known moist chamber, except that a constant supply of air is assured when needed, manipulation is much simpler,

*From the Department of Clinical Laboratories, Cleveland Clinic, Cleveland, Ohio.
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and the chances of contamination are appreciably lessened. The thinness of the cover-glass permits observation through the highest power.

If for any reason a solid medium is desired, as for the study of bacterial colonies, we have found it advisable to seal the cover slip to the *inner* surface of the dish, proceeding with the sterilization as described above. A very thin layer of melted medium, at as low a temperature as possible, is poured into the plate, and the inoculation is made over the site of the cover slip; or the tube of melted medium may be inoculated and then poured into the plate, with a strong probability that some of the colonies will occur at the site of the cover slips. Incubation is done as described above, observations being made at daily or more frequent intervals.

The Gorgas Memorial Institute

TWENTY-FIVE million cases of illness every year in the United States are a challenge to the whole medical profession, especially when it is considered that at least 20 per cent and perhaps 40 per cent of such illness is believed preventable.

The profession is aware of the havoc wrought by infections of the oral cavity, and appreciates that eradication of such infections means less illness. The cooperation of dentists with the Gorgas Memorial Institute is significant of an era in which the fight to conquer disease will be backed by a triple alliance—physician—dentist—individual.

The 35,000 dentists who are members of the American Dental Association have joined with the Gorgas Memorial Institute in its work of decreasing preventable illness and the consequent premature deaths which result from ignorance, carelessness, and lack of hygiene.

While the support of the American Dental Association is encouraging to the professions involved, such action also bodes well for the millions who harbor infections of the oral cavity. Many such persons need the services of the physician after their condition has been detected by the dentist. And by the same token, physicians will take precautions to see that their patients get the needed dental care which their condition demands.

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EDITORIALS

Dengue Fever

DENGUE fever which is endemic in our southern states not infrequently reaches the proportions of a terrific epidemic; its incidence and rapidity of extension exceeding even that of influenza. Chandler and Rice (1922) estimated that the epidemic of 1922, in Texas, claimed between 500,000 and 600,000 victims. Fortunately dengue is never fatal, but it is disabling and during the disease the patient suffers excruciatingly. As a cause of disability alone, even though we should consider the average days lost as only five, the cost of this one epidemic to the State of Texas was enormous. Recently there has been an epidemic in India, possibly five or more times the size of our own. In the Philippines, dengue affects chiefly the newcomers—natives seem to have a high degree of acquired immunity. The visitors affected are largely Army and Navy people, the families of those sent there for duty. Such persons sometimes come down with dengue within one week of their arrival in Manila.

Dengue is, therefore, a disease of no mean importance from a military standpoint. Its possible interference with emergency maneuvers could be most serious in any campaign involving the transfer of troops from regions where dengue does not usually exist, into an endemic center. It is not surprising, therefore, that among the problems taken up by the U. S. Army Medical Department Research Board in Manila, dengue has had an important place and the report of the work (1925) done upon it shows that several of the important points in the epidemiology and mechanism of transmission of dengue have been cleared up for all time, and our knowledge of the disease has been greatly enlarged.

The conditions under which the work was done made it possible to control those factors which have cast doubt upon previous dengue work along the same lines. The results, therefore, are clear cut and enough of the experimental details are given in the preliminary report to show that the work has fundamental value.

As experimental material, forty-two American soldiers volunteered for the transmission experiments and dengue was caused experimentally by the bites of mosquitoes in twenty-five instances.

It will be remembered that Graham, in 1903, first reported the transmission of dengue by *Culex* mosquitoes. It is evident that not all the mosquitoes in his experimental lot were *Culex* however, and his experiment was not well controlled.

It will also be recalled that Ashburn and Craig (1907) first showed that dengue can be transmitted by injecting the blood of a patient into a susceptible individual and that the cause of dengue is filtrable; they produced the disease through the injection of filtrates of the blood. In their mosquito transmission work, one of their cases indicated that *Culex* as well as *Aëdes* (*Stegomyia*) could transmit dengue but they have themselves wanted this confirmed, the conditions of the experiment not having been satisfactory to them. Cleland, Bradley and MacDonald (1918) demonstrated quite definitely that *Aëdes egypti* is a transmitter of dengue. The question as to the potential rôle of *Culex* therefore needed to be cleared up. The knowledge that *Aëdes* is able to transmit dengue needed to be amplified and further information concerning the exact mechanism of transmission was required before our information could be applied in prevention work.

All the mosquitoes used by the Board were bred from the eggs and in each experiment the exact number used was known; exact counts of those that actually took blood were made following each contact with a volunteer. The volunteer subjects of the experiment were kept in strict isolation in a special ward of the Sternberg General Hospital. The ward was entered through a series of doors in a screened vestibule and all screens were examined every day to be certain they were in perfect condition. The volunteers were held under observation for a sufficient period, before subjecting them to the bites of the mosquitoes, to be certain they were not already infected.

Dengue fever was transmitted by the bites of infected *Aëdes egypti* mosquitoes twenty-five times. The *Culex* failed to produce dengue in five patients under the same conditions and after waiting for a sufficient time to

cover any possible incubation period, the same persons were bitten by *Aëdes*—all then came down with dengue. *Culex quinquefasciatus,* therefore, is finally ruled out as a potential transmitter of dengue fever.

The dengue patient is able to infect mosquitoes during the twenty-four hours preceding the onset of symptoms and during the first three days of the disease, but not later. The virus seems to be present in the circulating blood in highest concentration on the first day of the disease.

Just as in yellow fever, the virus must remain in the mosquito eleven days before the bite becomes infective, but this period past, the *Aëdes* remains infective for a very long time—probably throughout the rest of its life.

Immunity studies indicated that one attack confers a certain degree of resistance but this gradually disappears. Second and even third attacks in the same individual are not at all uncommon and the relatively high immunity of natives can be accounted for best by the assumption that they are frequently reinfected.

With this exact information now at hand, it is possible to direct efforts at prevention along definite lines. The *Aëdes egypti* mosquito is a most difficult one to control with methods available at present but with studies directed specifically against it, who can say what the result may be? Patients should be screened from mosquitoes during the first three days of the disease. If this is done the incidence of dengue will certainly be reduced. The factor in epidemiology most difficult to control—impossible, in fact—is the infection of mosquitoes during the last twenty-four hours of the incubation period.

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—*A. P. H.*

Erratum

The title of the article by Graham and MacCarty on page 548 of the April issue of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE was printed incorrectly through an error not the fault of the authors. The words "Spinal Cord" should read "Spinal Fluid"—"On the Application of the Hench-Aldrich Urea Index to the Spinal Fluid."

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Practical Lectures**

THE volume is a compilation of papers presented before the Medical Society of the County of Kings, Brooklyn, N. Y. In every sense it lives up to its title. The contributors are predominately practicing physicians and surgeons who have obtained preeminence in their individual fields and who in their lectures have endeavored particularly to give points of practical importance and interest to the practicing physician. The titles of the individual papers give a good idea as to the character of their contents. Examples are: "The Surgical Abdomen" by Dr. Joseph A. Blake; "Surgical Diagnosis" by John B. Deaver; "Rational Pathology in the New Therapeutics" by J. W. Ewing; "The Common Skin Diseases" by John A. Fordyce; "Diagnosis of Abdominal Tumors" by Russell S. Fowler; "Diagnosis and Treatment of the More Common Recto-Colonic Diseases" by Samuel G. Gant; "Renal Function in Clinical Medicine" by Herman O. Mosenthal; "Everyday Bacteriology" by Wade W. Oliver; "Office Orthopedics" by Walter Truslow; "Fundamental Signs in the Examination of Patients" by Luther F. Warren. In all there are twenty-five contributions.

We cannot recommend this book too highly to practicing physicians as a reference volume covering recent advances in general diagnosis and treatment.

Goiter: Nonsurgical Types and Treatment†

WE find this book apropos in view of recent developments in the nonsurgical treatment of the various types of goiter. The author takes the stand that a large percentage of goiters surgically treated are perfectly amenable to nonoperative procedure. His primary purpose is to assist the general practitioner in classifying his cases into surgical and nonsurgical types, and to indicate what methods bring about the best and promptest results in those instances obviously of nonsurgical nature. Among the nonsurgical diseases of the thyroid, he mentions simple parenchymatous hypertrophy, colloid goiter, puberty hyperplasia, and the hyperplastic swelling of Graves' disease. Surgical goiter embraces adenoma, cystic goiter and other types of thyroid enlargement, not classified under nonsurgical goiter. In general, with the exception of strumitis, malignant goiter and diffuse adeno-

*Practical Lectures. Delivered under the Auspices of The Medical Society of the County of Kings, Brooklyn, N. Y. 132 illustrations. 3 color plates. Cloth. Price $5.50. Paul B. Hoeber, Inc., N. Y., 1925.

†Goiter: Nonsurgical Types and Treatment. By Israel Bram, M.D., Instructor in Clinical Medicine, Jefferson Medical College, Philadelphia. Illustrated. Pp. 479. Cloth. The Macmillan Co., 1924.

matosis, surgical goiters are encapsulated while nonsurgical goiters are diffuse or unencapsulated. An unencapsulated swelling of the thyroid gland implies a physiologic necessity for more thyroid hormone elsewhere in the economy or it indicates a defensive reaction against toxins during the existence of a focal or general infection. Encapsulation of a goiter is an indication that physiologic adaptation has ended and a pathologic reaction has begun. Pathologic or surgical goiters begin usually as physiologic or nonsurgical swelling, which, had they been treated at the proper time, would have been cured without surgery.

The major portion of the volume is devoted to exophthalmic goiter. The author summarizes the beneficial effect of iodine and stresses the use of quinine more than has been done recently by other authors. Many drugs are discussed pro and con. The author presents an excellent tabular differentiation between the various types of goiter.

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*Gonorrhea**

MIDWAY between the textbook, in which the author eliminates his own personality and the monograph in which he devotes practically his entire attention to his own work and theories, pausing to be sure, to compare these with the contributions of others, we find a group of contributions in which the author, while naturally giving major attention to his own work and theories, devotes a large amount of space to considerations of more general interest. The reader here finds himself possessed with an excellent general treatise on a particular subject, one which gives due credit to the various theories and experiments of other workers but which views the subject as it were through the spectacles of the author.

Dr. Thompson with the help of his associates has made a very distinct contribution to the literature of gonorrhea. The 500 pages are allotted as follows: 145 to bacteriology; 98 to anatomy, pathology, cytology, and clinical pathology; 40 to immunity and the complement-fixation test; 88 to vaccine therapy, serum therapy, chemical therapy, electro and thermo therapy; 14 to prophylaxis and abortive treatment, and 105 to clinical manifestations and the practical modern treatment of gonorrhea. This proportionate distribution gives a well balanced whole. Although the volume is a British contribution, the recently developed American methods of treatment receive due consideration.

Angina Pectoris†

WERE it not of tremendous intrinsic value, this work would even so be highly prized as the last contribution of the late Sir James MacKenzie, Dean among "family physicians" of the older school.

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*Gonorrhea. By David Thompson, O.B.E., M.B., Ch.B., Edin., D.P.H., Camb. With contributions by David Lees, D.S.O.; Claude H. Mills, M.R.C.S., Eng.; Robert Thompson, M.B., Ch.B., Edin; Kenneth Maclachlan, M. B., Ch.B., Edin. Illustrated. Pp. 519. Cloth. Price $12.75. Henry Frowde and Hodder and Stoughton, London.

†Angina Pectoris. By Sir James MacKenzie, M.D., F.R.S., F.R.C.P., LL.D., Ab. and Es. F.R.C.P.I. (Hon). Cloth. Pp. 253. Price $9.00. Henry Frowde and Hodder and Stoughton, London, 1923.

The work is a true monograph in which the author presents a summary of his own original researches and his conclusions drawn therefrom. He makes no attempt to discuss other theories and gives few references to the work of others. At the outset he points out that in reading the literature of angina pectoris, we can but be struck with the number of untrue assertions made. Hypotheses are put forward supported by meager evidence or even without evidence. Such hypotheses are often taken as truths, so much so that methods of treatment are based upon them. Throughout the monograph, Dr. MacKenzie attempts to make no statement without clearly indicating the reasons therefor. His many years devoted to a study of pain are applied to the consideration of pain in angina. He stresses the knowledge that a correct understanding of angina will not be reached until we have attained a correct understanding of heart failure. The mechanism causing pain in angina is present in all individuals and the pain is elicited as a result of disturbed reflexes. It is muscular in origin according to Dr. MacKenzie's conception.

The importance of evaluating prognosis is stressed and prognosis is discussed in great detail.

Probably of foremost interest is the enumeration of the multiplicity of possible causes for the symptom known as angina and the discussion of the pathologic changes associated with these causes.

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*Internal Secretion and the Ductless Glands**

INTO the midst of a riot of unsupported theory and fancy on the action of the glands of internal secretion, there appears a contribution by a leader in the study of endocrinology, a contribution which should serve as a valuable "brake" in the hyperenthusiasm of others. The book is devoted to a critical study of those experimental facts which have been definitely demonstrated and to a discussion of the conclusions which may logically be drawn therefrom without the advancement of new all-inclusive and all-explaining theories.

Particularly in the interaction between the various ductless glands does the author caution against error in interpretation. "If there has been in many instances undue haste in formulating theories of the functions of individual glands, much more has this been the case in regard to theories of interrelationship. It is not certain that the clinicians have been greater offenders than the laboratory workers."

Major attention is devoted to the work on the internal secretion of the adrenal bodies, the thyroid and parathyroid and the pituitary body, although the internal secretion of the reproductive organs is given considerable space.

Where no conclusive argument has been promulgated, the author contents himself with the recording of observations. This applies for example in a discussion of the hypothetic internal secretions from the kidney and prostate.

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*Internal Secretion and the Ductless Glands. By Swale Vincent, LL.D., D.Sc., M.D., M.R.C.S., L.R.C.P., F.R.S., (Edin.) Illustrated. Pp. 422. Cloth. Price $8.50. Edward Arnold & Co., London. Second edition.

*The Physiology of Exercise**

APPEARING as a volume in "The Physical Education Series" edited by R. Tait MacKenzie, the first portion deals with the general effects of exercise upon bodily functions. Exercise is divided into various types. The active type includes the exercises of speed, exercises of endurance, of strength, skill, attention, and alertness. Support type of exercises consists of exercises on the feet and in the head-down position. Passive types include massage and passive movement. Finally, the author discusses postural types of exercise. In the physiologic consideration, chief attention is perforce given to the circulation, the blood pressure and the respiration, although chapters are also devoted to the blood and the neuromuscular mechanism.

In the second part of the book, we find the practical application of the points brought out in the first section with divisions devoted to gymnastics, athletics, aquatics and to physical efficiency tests. The physiology of training as studied by the author in the training of large groups of athletes is discussed in detail.

The book comprises a practical application of the studies of the author as director of physical education at the International Y. M. C. A. College at Springfield, Mass. The scientific character and value of the contributions that have in the past come from this institution have not heretofore received the general recognition which they undoubtedly deserve.

Textbook on Biology†

A TEXTBOOK on general biology for collegiate students which aims to give a general recapitulation while avoiding too technical considerations. Comparative biology is presented as observed in the animal and vegetable kingdoms, followed by certain practical applications and deductions including the theories of heredity, a discussion of acquired characteristics, theories of evolution, the study of fossils and its application to the theory of adaptation, the geographical distribution and meaning of color, and the effects of environmental changes.

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*The Physiology of Exercise: A Textbook for Students of Physical Education. By James Huff McCurdy, A.M., M.D., M.P.E. Cloth. Pp. 242. Illustrated. Price $3.00. Lea & Febiger, Philadelphia, 1924

†A Textbook on Biology. For students, in General Medical and Technical Courses. By William Martin Smallwood, Ph.D. (Harvard.) Fifth edition, thoroughly revised. Illustrated with 249 engravings and 3 plates in colors. Pp. 393. Cloth. Price 3.75. Lea & Febiger, 1924.

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The Fourth Annual Convention of the American Society of Clinical Pathologists

Just as we go to press news has reached us of the great success of the American Society of Clinical Pathologists meeting held May 20-23 at Philadelphia. Close to 150 members signed the registration book. The enthusiasm was as great and as inspiring as characterized the Rochester conclave a year ago. All of the essayists, save one, who was incapacitated by illness, were present at the meeting to read their respective papers. Needless to say, the papers were of a high scientific character.

The new officers elected are as follows: President, Dr. Frederick Sondern, of New York; President-elect, Dr. William G. Exton, Newark, N. J.; Vice-President, Dr. F. L. Burnett, Boston, Mass.; Secretary-Treasurer, Dr. Ward Burdick, Denver, Colo. Member of the Executive Committee, Dr. Philip Hillkowitz, Denver, Colo. Member Board of Censors, Dr. Geo. Ives, St. Louis, Mo.

Full details of the meeting will appear in the next issue.